Effects of Increased Temperature on Growth and Seed Production of Soybean

2013

Custódio R. P. Tacaríndua

Contents

Summary

The anticipated increase in global surface temperature will have negative impacts on important field crops in the temperate region, including soybeans, which are important source of oil and protein. Although several experiments have been conducted on soybean, the information is not enough to quantify the effects of increased temperature on total biomass and yield. The temperature gradient chamber (TGC) allows a study of quantitative effects of temperature on crop performance under field-like conditions. But observation in TGC is limited and considerable inconsistencies exist regarding the response of growth and yield. Therefore the objective of this study was to investigate the effects of increased temperature on soybean growth and seed production using TGC.

Soybean cultivars (cvs.) Enrei (2009, 2010, 2011, 2012), Ryuho and Suzuyutaka (2009) were grown in TGCs to investigate the effects of increased temperature on growth and seed production under field-like conditions. Three temperature treatments, near ambient temperature (Ta), ambient temperature + 1°C (Ta + 1), and ambient temperature + 3°C (Ta+3, 2009 and 2010 only), and ambient temperature + 2°C (Ta + 2, 2011 and 2012 only), were established by dividing the rows along which the temperature gradient was created. Cvs. Ryuho and Suzuyutaka were grown under two temperature regimes and they were used to investigate only the response of seed growth characteristics to temperature. Single seed weight (SW) was expressed as a product of individual seed growth rate (SGR, mg $seed^{-1}$ d^{-1}) and effective filling period (EFP, d), determined by

periodic plant harvesting. The cotyledon cell number and cell volume were determined by maceration of seed samples.

Increasing mean air temperature by 2-3^{o}C in four study years resulted in reduced pod number (m^{-2}) and reduced seed number (m^{-2}) by 9-29% and 11-35%, respectively. Seed yield was reduced further by reduction of SW and seed number by 16 to 40%.

A temperature increase by 3^{o}C, about 26.4^{o}C in Ta vs. 29.4^{o}C in Ta+3 for the entire growth period, resulted in reduction of SW of Enrei by up to 14%, in 2009 and 2010. SGR was reduced by 19% on average, whereas EFP was increased by 3 days. Similar responses of EFP, SGR and SW to increased temperature were found in cvs. Ryuho and Suzuyutaka. Temperature increase (+3^{o}C) did not affect cell volume, but cell number was reduced by on average 35%. The results indicated that the temperature increase decreased the seed size of soybean by decreasing the cell number and SGR.

Increased temperature reduced aboveground dry matter (TDM) by 11-27%, and the harvest index (HI) by 6-32%. The responses of TDM and seed yield were greater than expected from the earlier studies. The reduction of TDM was associated with decline of photosynthesis and stomatal conductance. Analyses of change of vapor pressure deficit (VPD) and carbon isotope discrimination suggested that the concomitant increase of VPD with increased temperature exacerbated the temperature effects. In addition, reduced ambient CO_2 and low light intensity as the artifacts of the facility might have accounted for the great effect of temperature.

In addition, because high temperature and drought generally interact to affect plant growth, experiments were conducted in 2011 and 2012 to investigate the interactive effects of high temperature and drought under TGC conditions. In the drought TGCs soil

moisture content was maintained at about 15% as compared to 24% in the control. TDM and yield components were reduced by both increased temperature and drought. The reduction of TDM was associated with reduced stomatal conductance and photosynthesis. However, the effects of simultaneous occurrence of high temperature and drought were only additive. This was presumably due to the fact that the induced drought stress was moderate.

The temperature-related reductions of yield and dry matter are likely to occur in warm regions where high temperatures coincide with dryness of atmosphere. Estimated response using combined data from Tohoku region and Kyoto suggested that the optimum temperature for HI of cv. Enrei is about 26^{o}C. Increasing temperature by 2^{o}C above the optimum would result in 7% reduction of HI whereas a temperature increase by 3^{o}C would result in 13% reduction.

Given that the global warming is projected to continue, adjusting cropping system not to expose plants to hot environments in their sensitive stages as well as breeding cultivars for high temperature environment will help plants cope with the warming climate.

Chapter 1

Introduction

1.1 Soybean production and the global warming

Domesticated in the eastern half of north China around the 11th century B.C. (Hymowitz, 1970), cultivated soybean (*Glycine max* (L.) Merr) is an important food crop and its production has expanded to all of the populated continents, including warm regions, which are mostly characterized by high temperatures and low or erratic rainfall (Thuzar et al., 2010).

Soybeans are a major oilseed crop produced and consumed in the world, the second major vegetable oil after rapeseed and the most consumed protein meal among oilseed, with about 69% of total consumption in 2010/2011. The USA has long been the major soybean producer, and in 2010/2011 for example, they produced about 35% of the world total, followed by Brazil (27%), Argentina (19%), China (6%) and India (4%) (USDA, 2011). However, the world average of this important legume has not improved much in the last ten years, with world average yield of about 2.25 ton/ha in 2000-2003 (Martin et al., 2006) and 2.56 ton/ha in 2010/2011(USDA, 2013). It has been recognized that there is a need to increase the yield of soybean (Board and Kahlon, 2011) along with other food crops to keep pace with growing demand caused by growing populations and rising incomes (United Nations Environmental Programme, 2006).

With the loss of available land for food crops production to urbanization (Zhu et al., 2010) and to biofuel (Young, 2009; Zhu et al., 2010), it will be difficult to meet the world food demand through expansion of production area, as is the case of area for soybean production in the USA (Ainsworth et al., 2012). In addition, a recent assessment by the Intergovernmental Panel on Climate Change (IPCC) projected that the global average surface temperature would increase by a value between 1.4 and 5.8 °C this century (United Nations Environmental Programme, 2006), which could potentially have negative impacts on important agronomic crops, including soybean (Fedoroff et al., 2010; Hatfield et al., 2011).

Not only agronomic adaptation such as shifting cropping seasons but also development of heat-tolerant genotypes via conventional breeding or the use of advanced techniques of molecular breeding and genetic engineering are promising. A successful strategy, however, requires concerted efforts among different areas, including plant physiology (Wahid et al., 2007).

Indeed, several studies have been conducted to understand the response of physiological processes to increased temperature, but it seems that further studies are necessary in order to better quantify the impacts as well as characterize the exact mechanisms involved in the temperature response of biomass production and seed yield of soybean.

1.2 Temperature effects on soybean growth and development

Temperature, defined as the energy state of an object (Ormrod, 1978), is one of the principle controls over plant distribution and productivity. It has large effects on physiological activity at all spatial and temporal scales (Sage and Kubien, 2007) and has influence on time course of crop development. Time course of crop development, the ontogeny, is a continuity of the vegetative, the reproductive, the grain filling or maturation phases through which a crop completes one life cycle (Horie, 1994).

In soybean, the vegetative development is marked by the sequence of seedling emergence, cotyledons expansion, the appearance of first pair of opposite unifoliolate leaves and subsequent appearance of leaves that are alternate and trifoliate. After six or more trifoliate leaves have appeared, the floral initiation occurs (Martin et al., 2006). In the reproductive development, after pollination, pods begin to develop and reach an almost full width while the developing embryo is still very small. The seed filling begins and then seeds enter a period of rapid linear dry matter accumulation. Physiological maturity is reached when pods lose their green color and soybeans are harvested after drying in the pod to 15% (or less) moisture (Burton, 1997).

The rate of development in each phase, or inversely its duration, is determined by genetic and environmental interactions (Horie, 1994) among which, light, water and temperature. Temperature has effect on development, first as a general promoter of development through activation of enzymatic processes, and second as a modifier and a preconditioner of photoperiodism, as in vernalization (Horie, 1994).

Rates of development and progression through a crop life cycle may show linear responses to temperature (Porter and Semenov, 2005), which is implicitly assumed in the traditional degree-day or thermal-time model (Horie, 1994). However, the responses of developmental rate to temperature can be better described by a developmental index (DVI) model (Horie, 1994), which has been successfully applied to predict development toward flowering of nonvernalization-requiring genotypes of many crops, including soybean (Fig. 1). It can be seen in the figure that regardless of day length, as the temperature increases the rate of development toward flowering increases until an optimum mean temperature where the maximum developmental rate (DVR) is reached. However, despite being neglected in this model, the actual DVR should decrease with further increases of temperature above the optimum (Horie, 1994). As temperatures deviates from the optimal, physiological, biochemical, metabolic and molecular changes occur within plants (Guy et al., 2008), leading to severe retardation in growth and development, and even death (Kotak et al., 2007).

Time intervals between vegetative and reproductive events in soybean are known to be temperature dependent (Hesketh et al., 1972), with the optimum temperature differing between the developmental stages (Boote et al., 1998). The days from planting to cotyledon appearance, planting to unifoliolate, unifoliolate to trifoliolate and between trifoliolates, decreased with increased mean temperature from 13 to 30^{o}C. Similarly, low temperatures delay the rate of progress toward flowering (Hadley et al., 1984). On the other hand, the days from flowering to physiological maturity decreased with increased temperature from 22 to 30^{o}C (Hesketh et al., 1972). Thomas et al. (2010) also reported

delayed onset of reproductive stage by temperature above 27^{o}C, which appeared to be optimal for the soybean cultivar used in their experiment.

The rise in temperature above the optimum with negative impacts on plants is known as heat stress (Zrobek-Sokolnik, 2012) and is among the most commonly encountered stress factors, acting either independently or in combination with drought stress (Mittler, 2006; Kotak et al., 2007). With the current trend in surface temperature due to global warming, more extreme episodes of heat stress are likely to occur, thereby causing adverse impacts on crop yield.

There are several reasons why the rates of biological reactions do not continue to increase indefinitely with increasing temperature, but the most important is that most of them are enzyme catalyzed and temperature rise increase the rates of denaturation (Jones, 1992).

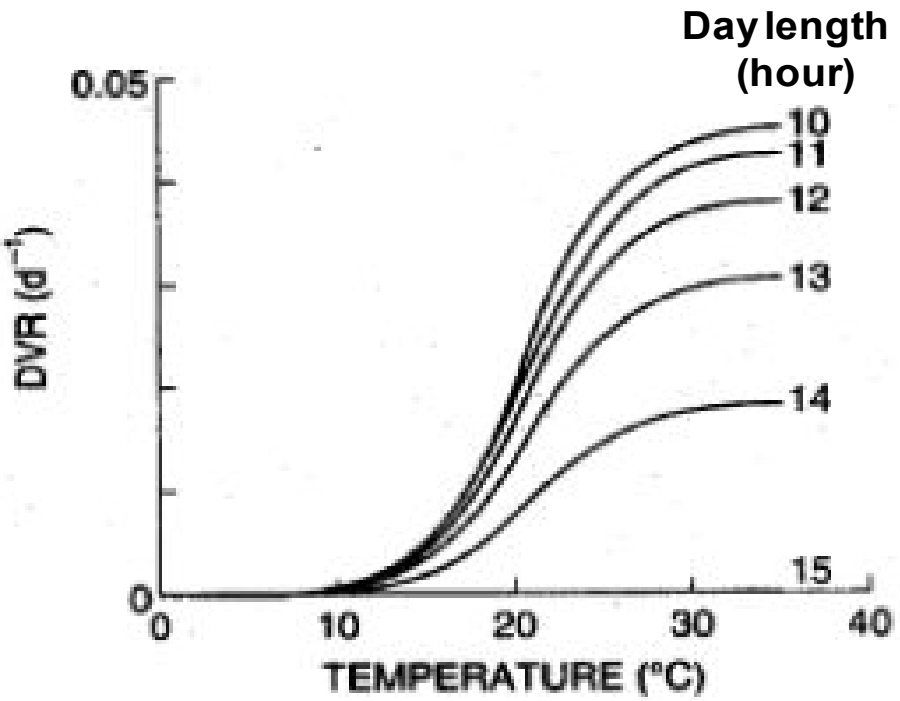

Figure 1. Rate of development of soybean cv. Fukuyutaka toward flowering as a function of day length and daily mean temperature, estimated by DVI model application to the experimental data (Horie, 1994).

1.3 Temperature effects on soybean yield and dry matter accumulation

Growing a crop is an exercise in energy transformation, which involves interception of incident solar radiation by the leaf canopy, conversion of the intercepted radiant energy to chemical potential energy (expressed in terms of plant dry matter, DM) and partitioning of the DM produced between the harvested parts and the rest of the plant (Hay and Porter, 2006). The yield (Y), per unit area, over a given period of time can be expressed by the equation:

$$Y=Q \times I \times \varepsilon \times HI$$

where:

Q is the total quantity of the incident radiation received over the period,

I is the fraction of Q that is intercepted by the canopy,

ε is the overall photosynthetic efficiency of the crop,

HI is the harvest index of the crop.

Yield can be influenced by temperature through effects on the fraction of intercepted radiation, radiation use efficiency ($I \times \varepsilon$), yield component formation and/or carbohydrate partitioning (Laffitte and Edmeades, 1997).

In previous studies, increasing mean air temperature resulted in smaller soybean seeds (Baker et al., 1989; Gibson and Mullen, 1996; Zheng et al., 2002; Mochizuki et al., 2005; Heineman et al., 2006; Thomas et al., 2010) increased seed size (Egli and Wardlaw, 1980; Sionit et al., 1987) or had no effect on the seed size (Sionit et al., 1987). In terms of seed yield, increasing temperature resulted in decreased yield (Gibson and Mullen, 1996;

Mochizuki et al., 2005; Heineman et al., 2006), increased yield (Sionit et al., 1987) slightly affected the yield (Ferris et al., 1999) or did not affect the yield of soybean (Zheng et al., 2002).

Increasing mean air temperature up to 32.5^{o}C resulted in decreased total biomass in soybean (Baker et al., 1989). Similarly, plants grown under high mean air temperature up to 34^{o}C from 10 days after sowing (DAS) to maturity had less total biomass than the control (Koti et al., 2007). However, biomass was not considerably affected by increased temperature of up to 39^{o}C (Allen and Boote, 2000) and HI was reported to be decreased by increasing mean air temperature beyond 26^{o}C (Boote et al., 2005).

Some of these differences in responses observed in previous studies might be due to the temperature ranges used and/or the developmental stage at which temperature treatments were applied. Furthermore, in most of these studies temperatures were held constant throughout the entire growing period or during a specific developmental stage. The magnitude of heat stress is known to depend upon the intensity (temperature in degrees), duration, the rate of increase in temperature (Sung et al., 2003; Wahid et al., 2007) and the stage of crop development (Prasad et al., 2008) with the reproductive stage being more sensitive than the vegetative. Heat stress event during pollination, for example, can severely reduce yield even when the seasonal average is within a favorable range (Long and Ort, 2010).

Given that temperature exerts control on growth and development of plants and the global warming is expected to continue, understanding the physiological mechanisms of plant response to temperature is crucially important, and results from field-like conditions are needed.

1.4 Temperature gradient chambers and researches on global warming

Rising CO_2 levels has the potential to benefit crop growth through stimulation of photosynthesis in most crop plants, but the links are complex (Porter, 2005). There are many additional climatic factors that may offset the positive impacts of rising CO_2 on plant growth (Hatfield et al., 2011), including temperature as a result of global warming. Long and Ort (2010) analyzed different scenarios of expected effects of climatic changes (increased temperature, increased CO_2 and droughts) and they concluded that the overall scenario is uncertain.

Studies about the effects of increased temperature or its interaction on soybean production have shown varied results regarding the response of biomass or photosynthesis response. In addition, the studies were conducted in various types of facilities, including those where the temperature and/or relative humidity are held constant for a certain growth period or throughout the entire plant life cycle, which are rare in the field condition. For the generalization of the temperature effect on soybean performance, it is necessary to characterize the temperature response of the individual processes that comprise the seed yield formation as well as estimate their relative contribution on the final seed yield and total dry matter production in an environment as close as possible to the field conditions. Temperature gradient chamber (TGC, Fig. 2) allows the study of temperature effects on crops under field like conditions, where the inside temperatures tend to track the diurnal ambient temperature (Horie et al., 1995).

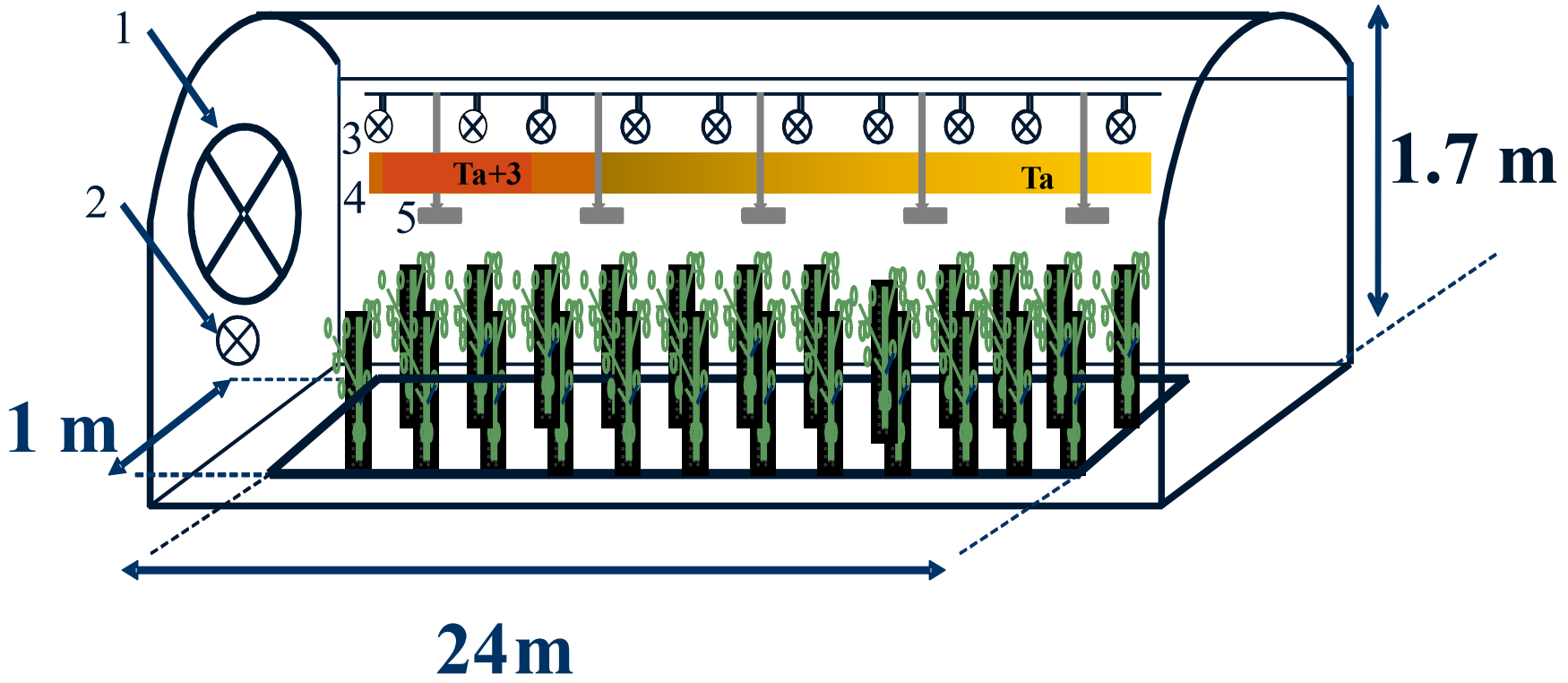

Figure 2. Schematic representation of TGC (2009 and 2010). 1-variable exhaust fan, 2-constant ehaust fan, 3-oscilating fan, 4-line heater, 5-aspirating thermocouples.

1.5 Interactive effects of high temperature and drought stress

Plants are generally exposed to multiple environmental stress, with drought and heat stress representing an excellent example of two different abiotic stress conditions that occur simultaneously in the field (Mittler, 2006). They are between the two most important environmental factors influencing crop growth, development and yield processes (Prasad et al., 2011). Despite their simultaneous occurrence, these two stresses are usually studied independently (Machado and Paulsen, 2001; Prasad et al., 2008) although their combined effects may be different from the effects when applied independently (Mittler, 2006).

It has been recognized that there is need for change of focus in plant stress research, in order to allow the development of plants that are resistant to multiple stresses yet maintain high yield (Atkinson and Urwin, 2012).

Combination of high temperature and drought stress have been studied in some grass, including wheat, barley and Ketucky bluegrass, where they were found to have higher detrimental effects on biomass, yield components and physiological and biochemical processes than each stress applied separately. Higher detrimental effects of high temperature and droght combined as compared to their effects separately have been reported for yield (Shah and Paulsen, 2003; Xu and Zhou, 2006; Prasad et al., 2011; Pradhan et al., 2012), aboveground biomass (Shah and Paulsen, 2003; Prasad et al., 2011), grain size (Shah and Paulsen, 2003) and grain number (Prasad et al., 2011). Similar effects were reported for photosynthesis (Shah and Paulsen, 2003), water relations (Machado and Paulsen, 2001; Grigorova et al., 2011) and leaf chlorophyll content

(Prasad et al., 2011). At the biochemical level, the induction of heat shock proteins is generally regarded to be higher under combined drought and heat stress, as compared to the induction observed under individually applied heat or drought stress (Grigorova et al., 2011).

Despite the commonly reported synergistic effects of high temperature and drought, additive effects can occur depending on the temperature and/or drought levels. In *Arabidopsis*, for instance, moderate levels of stress were found to have additive effects on plant biomass and physiological parameters (Vile et al., 2012).

Understanding the interactive effects of heat and drought stress on plants not only is important for the current environmental conditions encountered in the field but also for the future climate that is projected to be characterized by frequent episodes of high temperatures and reduced precipitation (Hartfield et al., 2011). Furthermore, studies on plant responses conducted under conditions that relate closely to natural or field conditions are highly recommended (Atkinson and Urwin, 2012) but they are rare.

1.6 The objectives of the study

It is desirable to quantify the impact of increased temperature on globally important crops like soybean, as the global warming is considered to continue.

The objective of this study was to investigate the effects of increased temperature on soybean growth and yield production using temperature gradient chambers (TGC). In addition, the interactive effects of increased temperature and water deficit on soybean growth and yield were investigated.

The study of interactive effects of drought ad heat stress will contribute with more information on the matter, given that there is limited information on their interaction despite their simultaneous occurrence (Prasad and Staggenborg, 2008) and it has rarely been studied under field-like conditions. In the future climate not only temperatures are projected to increase, but also the precipitations are expected to decrease.

Chapter 2

Effects of increased temperature on seed number and seed size

2.1 Introduction

The soybean yield is determined basically by the number of flowers a plant can produce, the percentage of those it can develop into mature seed, and seed size (Van Schaik and Probst, 1958). Although yield is more closely associated with seed number (Sadras and Egli, 2008) and most cultural and environmental factors influence yield through differences in seed number per area (Board and Modali, 2005), variations in individual seed weight can still account for differences in yield between environments (Munier-Jolain and Ney, 1998). Heat stress is known to influence yields through seed numbers but can also directly influence seed-filling duration, leading to smaller seed size and lower yields (Prasad et al., 2008).

In soybean, increased temperature reduced the number of fertile pods and the number of seed per plant, especially when high temperatures occurred prior to the beginning of seed filling period (Gibson and Mullen, 1996). Early embryo development is among the highly susceptible processes to heat and the failure can lead to embryo abortion and result in lower number of seeds and limit crop yield (Prasad et al, 2008).

Plants grown at mean temperature of 27.5^{o}C had fewer seed as compared to plants grown at mean temperature of 17.5^{o}C (Heinemann et al., 2006).

Individual seed size, which is the product of individual seed growth rate (SGR) and the duration of seed filling (Munier-Jolain and Ney, 1998; Sadras and Egli, 2008; Prasad et al., 2008), was decreased by temperature incase from 25 to 30 °C from the time of flowering (Zheng et al., 2002). A similar response was reported by Gibson and Mullen (1996), when temperatures were increased from 30.5 to 32.5 °C at any stage after flowering. Thomas et al. (2010) analyzed the effect of elevated temperature on the seed growth of soybeans and noted that growing soybeans at supra-optimal temperatures delays post-anthesis reproductive processes and the onset of seed growth, decreases the SGR and increases seed shriveling.

These findings are important and may be applicable to various situations, including the moderately increased temperature caused by year-to-year variations in the weather. However, temperatures are rarely constant throughout the entire growing season or during a specific developmental stage under field conditions, and, to date, few studies have been conducted to investigate the soybean seed number and seed growth response to temperature under field-like conditions. Furthermore, the exact mechanisms involved in the response of seed growth to temperature are not clearly understood.

In this study, the objective was to investigate the effects of increased temperature on soybean growth and yield formation using temperature gradient chambers (TGC) that mimic field conditions.

2.2 Materials and Methods

2.2.1 Materials and environment description

Soybean cultivars Enrei (from 2009 to 2012), Ryuho and Suzuyutaka (in 2009 only), determinate and of maturity group IV, were grown under various temperatures in TGCs (Fig. 2) at the Experimental Farm of Kyoto University at Kyoto City, Japan (35.0°N lat., 135.5°6′E long., 71 m asl). The TGC, which was 2 m wide and 25 m long, created a nearly linear temperature gradient along its longitudinal axis from near ambient to a temperature that was several degrees higher, while maintaining the natural diurnal changes in air temperature (Horie et al., 1995). The chambers were covered with polyethylene terephthalate film with a light transmittance of 80%.

Soybean seedlings were transplanted on July 13 and July 21 in 2009 and 2010, respectively, and directly sown on July 12, in 2011 and 2012 into the soil culture bed in two TGCs, arranged in four rows of 0.25 m wide, 24 m long, and 0.25 m intra-row spacing. Three temperature treatments, near ambient temperature (Ta), ambient temperature + 1°C (Ta + 1), and ambient temperature + 3°C (Ta + 3, in 2009 and 2010 only), and ambient temperature + 2°C (in 2011 and 2012 only), were established by dividing the rows along which the temperature gradient was created. In 2009, there were three additional plots between Ta and Ta+3 °C, namely, Ta', Ta+1' and Ta+2', in which cultivars Ryuho and Suzuyutaka were planted.

The TGC was equipped with an irrigation system containing an irrigation pipe located 50 cm below the soil surface. Water was supplied through the pipe to raise the water table to approximately 30 cm below the soil surface, and the water was evenly

distributed throughout the entire soil culture bed. The moisture content was monitored in 2010 using a moisture sensor (ECH_2O EC-5 moisture sensor; DECAGON, Pullman, Washington, USA) installed at a depth of 15 cm, whereas in 2011 and 2012, soil moisture content was monitored using a time domain reflectometry (TDR) meter (SONY Tektronix Co. Ltd., Tokyo, Japan) installed at a depth of 30 cm. The volumetric water content was maintained at approximately 22.2% and 24.2% (in 2009 and 2010, respectively) and 24% (in 2011 and 2012) throughout the entire growth period by sub-soil irrigation. Considering that the range of soil water content for plant transpiration was considered to be between 33% (field capacity) and 13% (data not shown), these values appear to be approximately one-half the level of transpirable soil water. The soil used is classified as alluvial sandy loam (Fluvic Endoaquepts) and the plots were kept weed free by chemical control or hand weeding.

2.2.2 Measurements

In all four years dates of the developmental stages (Fehr and Caveness, 1977) were recorded, seed growth attributes were analyzed only in 2009 and 2010 (for cv. Enrei) and in 2009 (for cv. Ryuho and Suzuyutaka).

(1) Determination of seed growth attributes

For seed growth attributes, the aboveground parts of two plants per replicate were sampled at roughly a one-week interval from the beginning of R5 to the beginning of maturity (R7). The temperature environment was not uniform within each treatment plot,

with the temperatures at one end close to the next treatment plot due to the nature of the TGC. To minimize the experimental error caused by this absence of uniformity within a plot, samples were always collected from both the cooler and warmer halves of the plot area, using one plant from each section. All of the complete seeds were collected and oven-dried separately from other plant organs for more than 72 hours at 80 °C to determine the dry weight. The completely shriveled seeds, which stopped growing (Fig. 3), were not separated from the pod shells, and the weight was included in the pod shell weight. The mean single-seed weight was determined by dividing its dry weight by the number of seeds. The SGR was determined as the linear coefficient of the single-seed weight vs. time (d), as based on the periodic measurement of seed weight during the linear phase of seed filling. The effective seed-filing period (EFP) was calculated by dividing the final single-seed weight at maturity by the SGR (Fig. 4) (Egli and Wardlaw, 1980).

(2) Determination of cotyledon cell number and cell volume

For the determination of cell number and cell volume, five seeds from samples harvested at maturity (three replications) were collected from three treatments for each TGC. To determine the cotyledon cell number and cell volume, the procedures of Sexton (1997) were followed, with minor modifications. Briefly, after maceration, the samples were diluted 10 times, and 15 μL aliquots were loaded into the sample injector of a hemacytometer (C-Chip, Disposable Hemocytometer, Fuchs-Rosenthal, Seoul, Korea). The estimates of the cell number per subsample were performed according to Sexton (1997), and the cells per volume were multiplied by the dilution factor.

(3) Calculation of DM and yield components

At harvest maturity, plant components of eight plants per treatment were separated into leaves (including petioles), stems, pod shells, and seeds that were used to calculate the final single-seed size, seed number, seed yield, and total aboveground DM.

2.2.3 Statistical analysis

The effects of increased air temperature on seed yield, yield components, DM accumulation, and carbon isotope discrimination (CID) were evaluated using an analysis of variance (ANOVA) with statistical software SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and CropStat.

Figure 3. The seed classification used in the present study. The completely shriveled seeds (dormant and unfertile) were not separated from the pod shells.

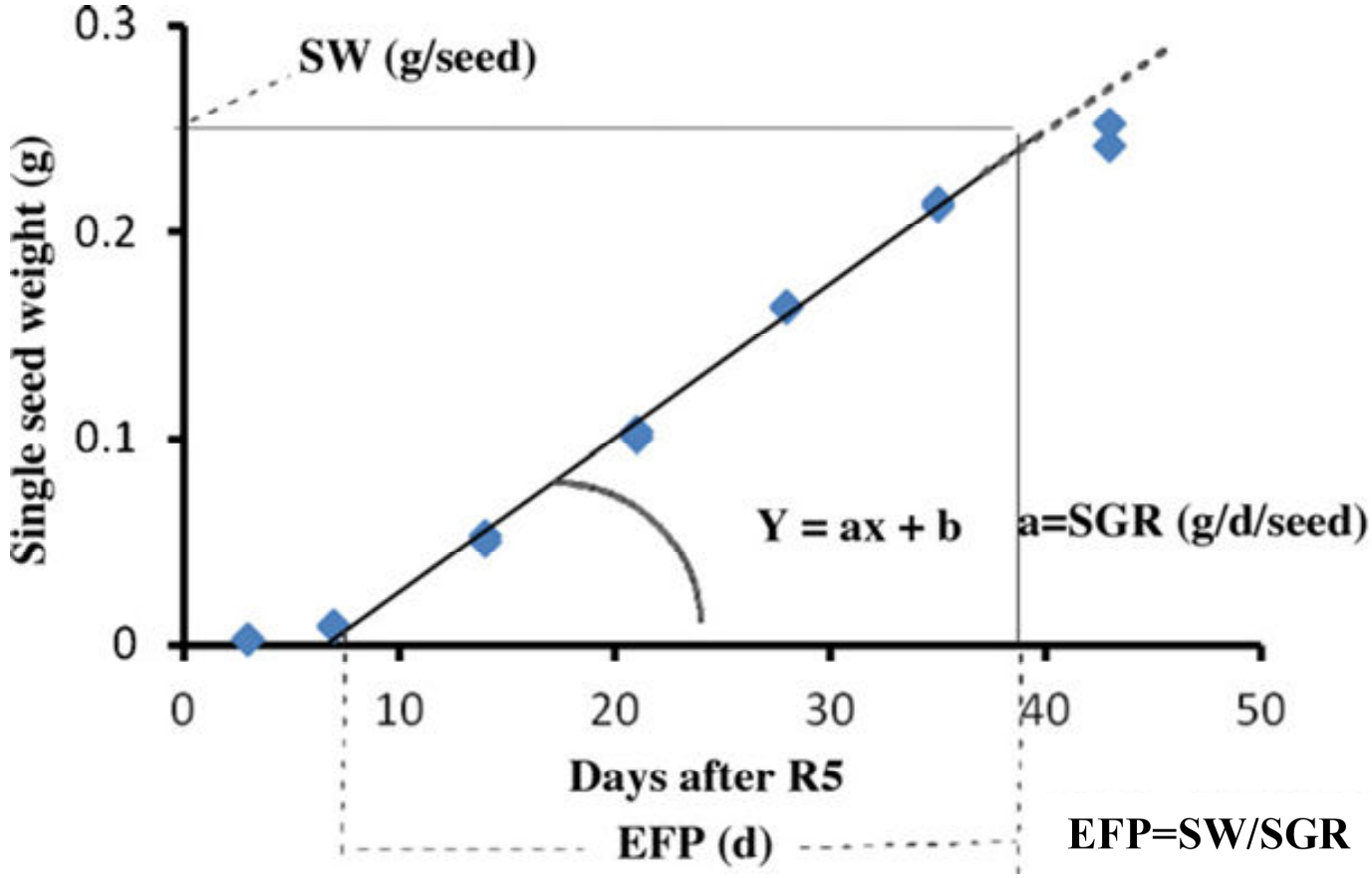

Figure 4. Calculation of SGR and EFP (SW from Ta in 2010)

2.3 Results

2.3.1 Temperature environment and vapor pressure deficit

The day, night, and mean air temperatures for the entire growing season are shown in Table 1. In the TGC, the lower temperature treatment was near the ambient temperature, and a temperature difference of approximately 2 to 3°C was achieved for the day, night, and mean temperatures. Changes in the daily mean temperature during the entire growing season are shown in Fig. 5. The inside temperatures tended to track the diurnal fluctuation of the outdoor temperatures. As generally happens in soybean production in this region, the plants experienced a period of relatively high temperature from R1 to R5 in the four study years. The temperatures tended to decrease in the mid-seed filling period and then increased slightly toward physiological maturity (R7). This was followed by relatively low temperatures until harvest maturity.

Estimated day, night, and mean vapor pressure deficit (VPD) from emergence (VE) to R7 are shown in Table 1. In general, most data for daily mean VPD ranged from 0.82 to 1.37 kPa in 2009, and from 1.40 to 2.13 kPa in 2010. In 2011 and 2012, the daily mean VPD ranged from 0.9 to 1.5 kPa and the day average reached approximately 2.1 kPa before R1. The VPD tended to be higher in the warmer treatment compared to that under Ta, and if we compare the four study years, the VPD in 2010 was the highest of all years.

Table 1. Vapor pressure deficit as affected by increased temperature

Year	Air temperature			VPD (kPa)								
				VE-R1			R1-R5+10			R5-R7		
	Mean °C	Day °C	Night °C	Mean	Day	Night	Mean	Day	Night	Mean	Day	Night
2009												
Ta	25.9	28.2	23.7	0.82	1.17	0.47	1.09	1.55	0.63	-	-	-
Ta+1	27.1	29.3	25.0	1.11	1.44	0.79	1.27	1.69	0.85	-	-	-
Ta+3	28.7	31.1	26.2	1.37	1.71	1.02	1.36	1.72	1.00	-	-	-
2010												
Ta	27.1	29.5	24.7	1.52	2.12	0.93	1.40	1.90	0.91	-	-	-
Ta+1	28.3	30.7	26.0	1.80	2.34	1.25	1.62	2.03	1.20	-	-	-
Ta+3	30.1	32.6	27.6	2.13	2.68	1.58	1.95	2.30	1.59	-	-	-
2011												
Ta	25.7	27.9	23.4	1.29	1.85	0.72	0.84	1.15	0.54	0.91	1.31	0.52
Ta+1	26.6	29.0	24.2	1.41	1.98	0.85	0.90	1.18	0.62	0.96	1.35	0.57
Ta+2	27.4	29.7	25.1	1.56	1.99	1.13	1.13	1.39	0.88	1.24	1.60	0.88
2012												
Ta	26.9	29.3	24.6	1.25	1.82	0.69	0.93	1.28	0.57	0.87	1.28	0.46
Ta+1	27.5	30.0	25.0	1.41	2.00	0.83	1.11	1.49	0.72	1.01	1.49	0.54
Ta+2	28.8	31.5	26.0	1.54	2.16	0.92	1.24	1.65	0.83	1.16	1.65	0.67

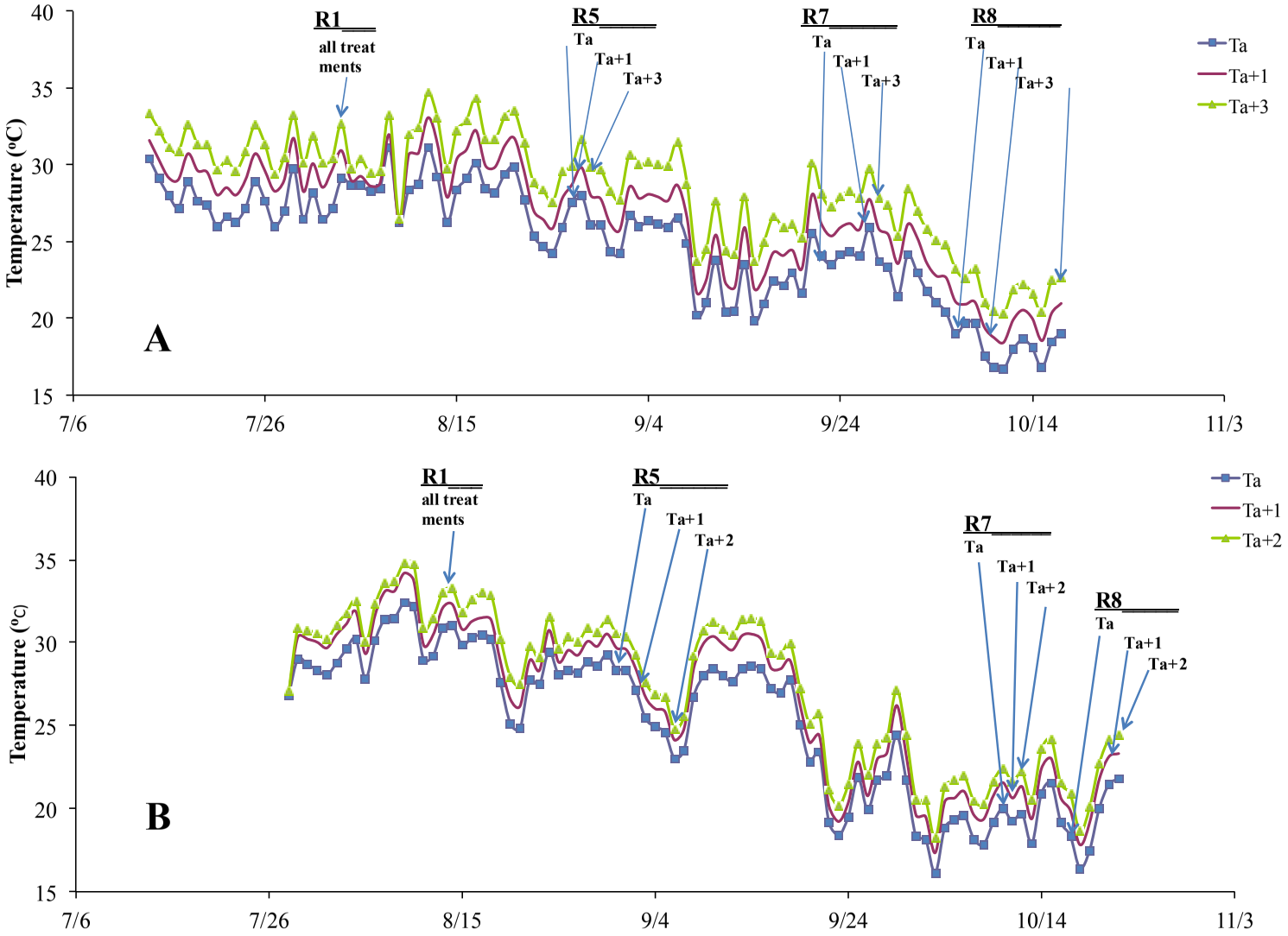

Figure 5. Daily changes in mean temperature in TGC, in 2009 (A) and 2011 (B)

2.3.2 Developmental stages

Increasing air temperature by approximately 2–3°C did not have a significant effect on R1, except in 2010, when flowering was delayed by 3 days. The number of days from R1 to R5 and the duration of seed filling (R5–R7) were significantly increased by increased temperatures (Table 2). The progress of pod setting (R1–R5) was more affected by the increase in temperature than the progress from VE to R1 and R5 to R7, particularly in 2009 and 2010. During these years, the time from R1 to R5 increased by 5 days, whereas the time from R5 to R7 increased by 2 days. There was a significant difference in the duration of the developmental stages among different years.

The delay in pod setting can also be seen in Fig. 6, which shows the progress of pod development expressed by pod DM to total aboveground DM plotted against days after the R5 date under the Ta treatment in 2009 and 2010. As the conditions were warmer, pod development was late at early R5, and this was held over to late seed filling in both years.

Table 2. Response in phenology to increased temperature

Year and Treatment	Days from		
	VE-R1	R1 to R5	R5 to R7
	(d)	(d)	(d)
2009			
Ta	30	27	35
Ta + 1	30	28	35
Ta + 3	30	32	37
2010			
Ta	30	14	39
Ta + 1	32	14	40
Ta + 3	33	19	41
2011			
Ta	27	19	40
Ta + 1	27	22	40
Ta + 2	27	24	38
2012			
Ta	25	19	37
Ta + 1	25	20	38
Ta + 2	25	23	39
ANCOVA	F value (P)		
Temperature (T)	13.28 (<0.01)	91.57 (<0.01)	14.30 (<0.01)
Year (Y)	2.5 (<0.1)	1.86 (n.s.)	10.07 (<0.01)
T x Y	4.93 (<0.05)	1.42 (n.s.)	8.27 (<0.01)

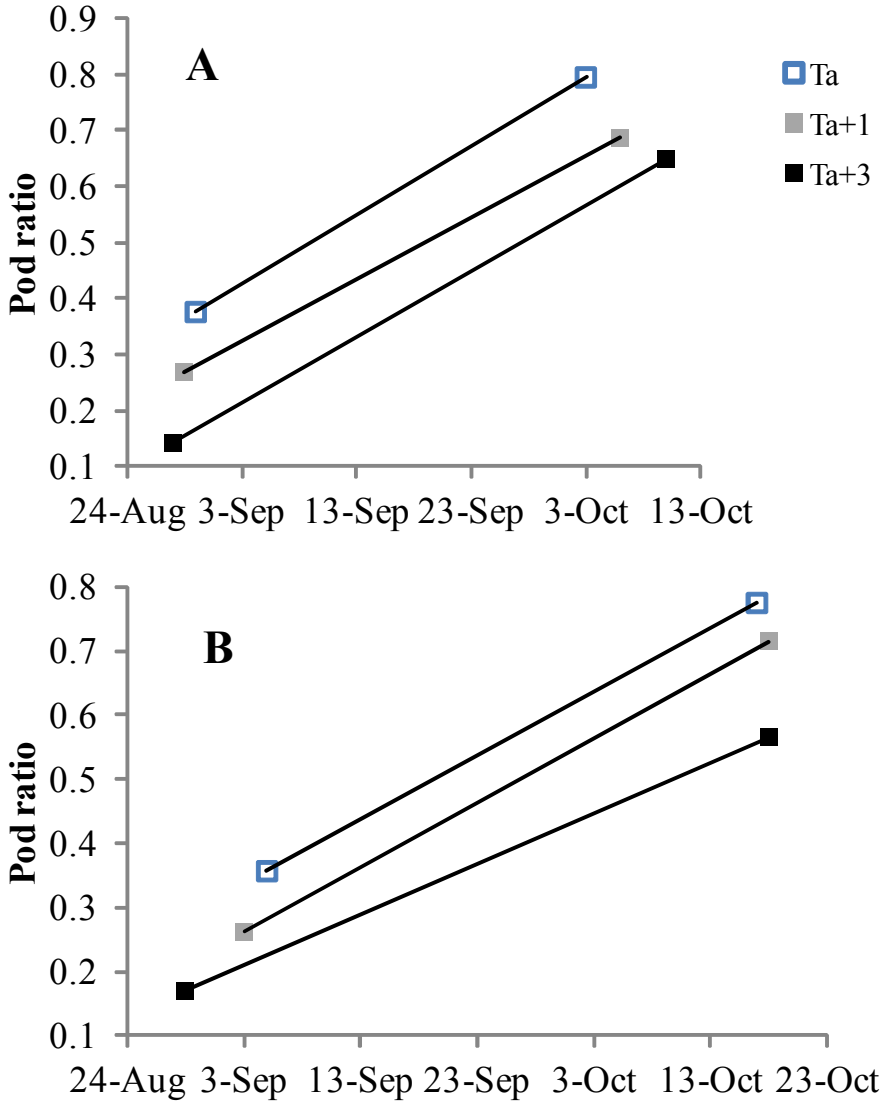

Figure 6. Pod dry weight per total dry weight at R5 + 5 (A, 2009), R5 + 10 (B, 2010).

2.3.3 Seed yield and yield components

Seed yield and yield components for all 4 years are presented in Table 3. Increased temperature had no significant effect on the number of nodes but significantly reduced the number of pods and seeds, seed size, and seed yield. The temperature increase reduced pod number by 30% and 16%, in 2009 and 2010, respectively, and by 10% in 2011 and 2012. Seed number was reduced by 35%, 28%, 22%, and 11% in 2009, 2010, 2011, and 2012, respectively. Single-seed weight reduction ranged from 5% in 2012 to 14% in 2010. Seed yield was most responsive to the increase in temperature, except in 2012, when it was reduced by 16%. In 2009, seed yield was reduced by approximately 40%, while in 2010 and 2011, it was reduced by 38% and 30%, respectively.

2.3.4 Single-seed size and seed growth attributes

Single-seed weight and related characteristics are presented in Table 4 only for cv. Enrei. The final single-seed weight, on a dry matter basis, ranged from 227 to 248 mg in 2009 and 208 to 242 in 2010. In 2009, the 3 °C increase reduced the single-seed weight by approximately 9%, whereas the reduction was 14% in 2010. There was no significant effect of the year or interaction between the treatment and year. Some seeds from the Ta+3 treatment were slightly shriveled (Fig. 3), and there appeared to be more shriveled seeds in 2010 than in 2009 (data not shown).

Table 3. Response of yield components to increased temperature

Year and Treatment	Yield	Node number	Pod number	Seed number	Seed number	Single seed weight[1)]
	(g m^{-2})	(/plant)	(m^{-2})	(m^{-2})	(/pod)	(mg)
2009						
Ta	403	28.1	879	1626	1.8	248
Ta + 1	305	27.6	732	1318	1.8	236
Ta + 3	241	34.9	628	1060	1.7	227
2010						
Ta	327	25.1	774	1349	1.7	242
Ta + 1	278	24.3	709	1202	1.7	231
Ta + 3	203	27.7	651	977	1.5	208
2011						
Ta	483	35.1	983	1844	1.9	262
Ta + 1	416	36.4	1005	1717	1.7	242
Ta + 2	336	29.8	893	1442	1.6	233
2012						
Ta	435	37.6	910	1641	1.8	248
Ta + 1	407	35.0	895	1589	1.8	240
Ta + 2	365	33.9	818	1458	1.8	235
ANOVA						
Temperature (T)	171.95 (<0.01)	0.95 (n.s.)	29.38 (<0.01)	220.04 (<0.01)	0.56 (n.s.)	13.75 (<0.01)
Year (Y)	5.07 (<0.01)	3.81 (<0.05)	1.11 (n.s.)	6.54 (<0.01)	0.93 (n.s.)	0.50 (n.s.)
T x Y	5.15 (<0.05)	3.49 (<0.05)	1.23 (n.s.)	6.81 (<0.01)	1.03 (n.s.)	0.50 (n.s.)

[1)] Based on dry matter

Table 4. Analysis of seed growth attributes of soybean cv. Enrei, as affected by air temperature.

Year and Treatment	Single seed weight[1)] (mg)	Seed growth rate1) (SGR) (mg d^{-1} $seed^{-1}$)	Effective filling period (EFP) (d)	Cell number (/cotyledon) ($x10^{-6}$)	Cell volume (nL)
2009					
Ta	248	8.5	29.4	5.0	0.200
Ta+1	236	8.0	29.5	4.4	0.196
Ta+3	227	7.1	32.2	3.4	0.180
2010					
Ta	242	7.5	32.5	4.3	0.196
Ta+1	231	6.7	34.5	3.5	0.234
Ta+3	208	5.9	35.2	2.6	0.277
ANOVA	F value (P)				
Treatment (T)	5.09 (<0.1)	19.86 (<0.01)	6.3 (<0.05)	51.1 (<0.001)	1.16 (n.s.)
Year (Y)	1.86 (n.s.)	35.8 (<0.01)	36.7 (<0.01)	34.69 (<0.01)	7.14 (<0.05)
T ×Y	0.4 (n.s.)	0.2 (n.s.)	1.1 (n.s.)	0.2 (n.s.)	3.27 (n.s.)

[1)] Based on dry matter

The SGR ranged from 7.1 to 8.5 mg per day per seed in 2009 and from 5.9 to 7.5 in 2010 (Table 4). In contrast, the effective filling period (EFP), which was calculated by dividing the final single-seed weight by the SGR, ranged from 30 to 33 days in 2009 and 33 to 35 days in 2010 and was significantly longer under higher temperatures; regardless, there was no interaction between the treatment and year. A similar response was observed in the final single-seed weight (Fig. 7A), SGR (Fig. 7B) and EFP (Fig. 8) for Ryuho and Suzuyutaka.

As indicated in Fig. 7A, the single-seed weight decreased as the air temperature increased in the range of approximately 26 to 30 °C for the three cultivars. The SGR also decreased as the air temperature increased, and there were linear trends of temperature responses for all three cultivars and both years for Enrei (Fig. 7B). This linear trend was notably consistent between 2009 and 2010 for Enrei for which the overall temperature range was higher in 2010 than in 2009.

Increasing the temperature significantly reduced the number of cells per cotyledon (Table 8), which ranged from 3.4 x 10^6 to 5.0 x 10^6 in 2009 and from 2.6 x 10^6 to 4.3 x 10^6 in 2010. These values are within the range reported in previous studies by Munier-Jolain et al. (2008), Thomas et al. (2010) and Liu et al. (2006).

The cell volume ranged from 0.180 to 0.200 nL in 2009 and from 0.196 to 0.277 nL in 2010. Unlike the observations for the cell number, the cell volume was not consistently affected in the two years. In 2009, the volume per cell showed slight reductions with increased temperature, whereas the opposite trend was observed in 2010. However, there was no significant difference among the temperature treatments or interaction between the treatment and year.

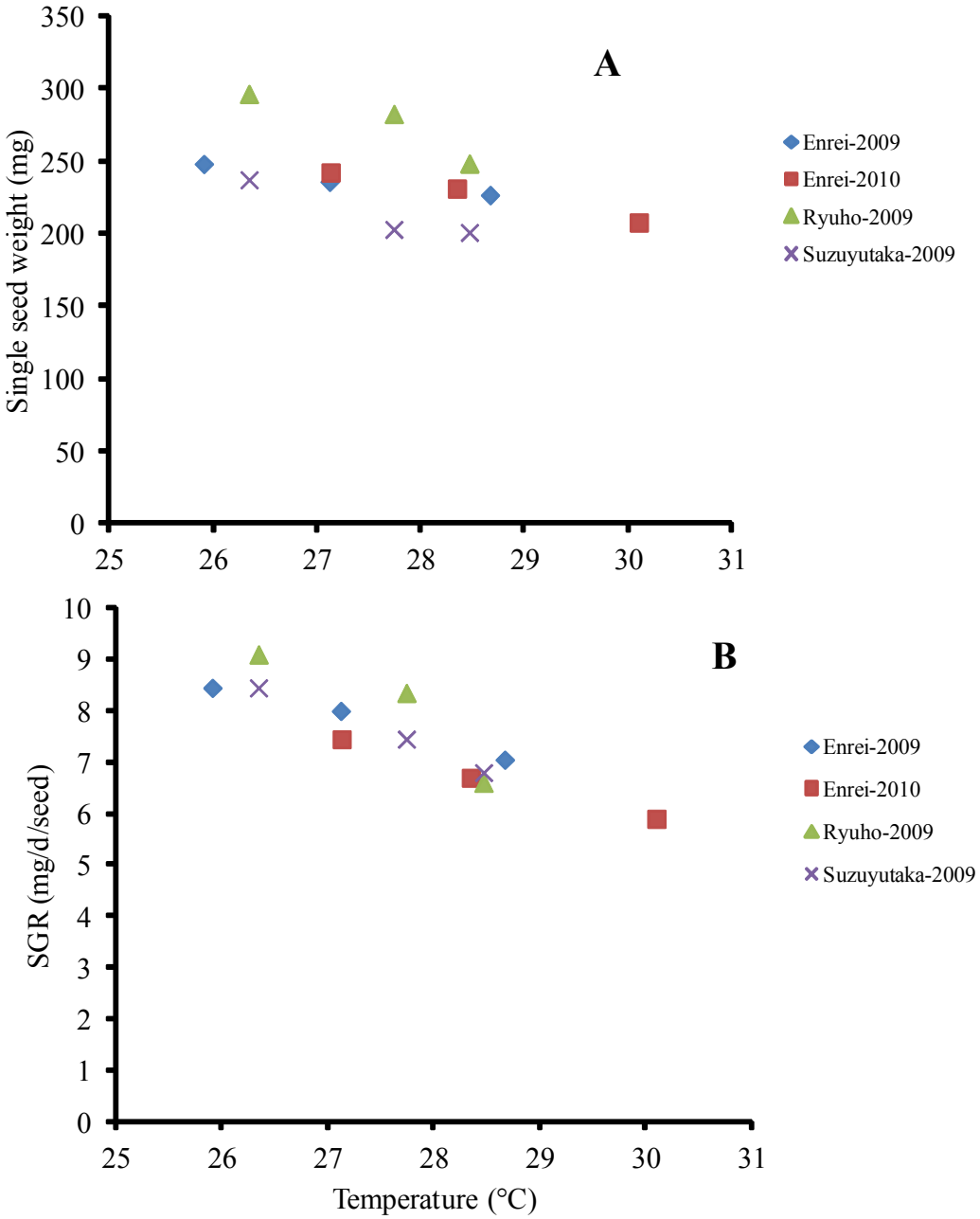

Figure 7. Seed dry weight (A) and seed growth rate (B) of soybean, as affected by air temperature (for the entire growing season).

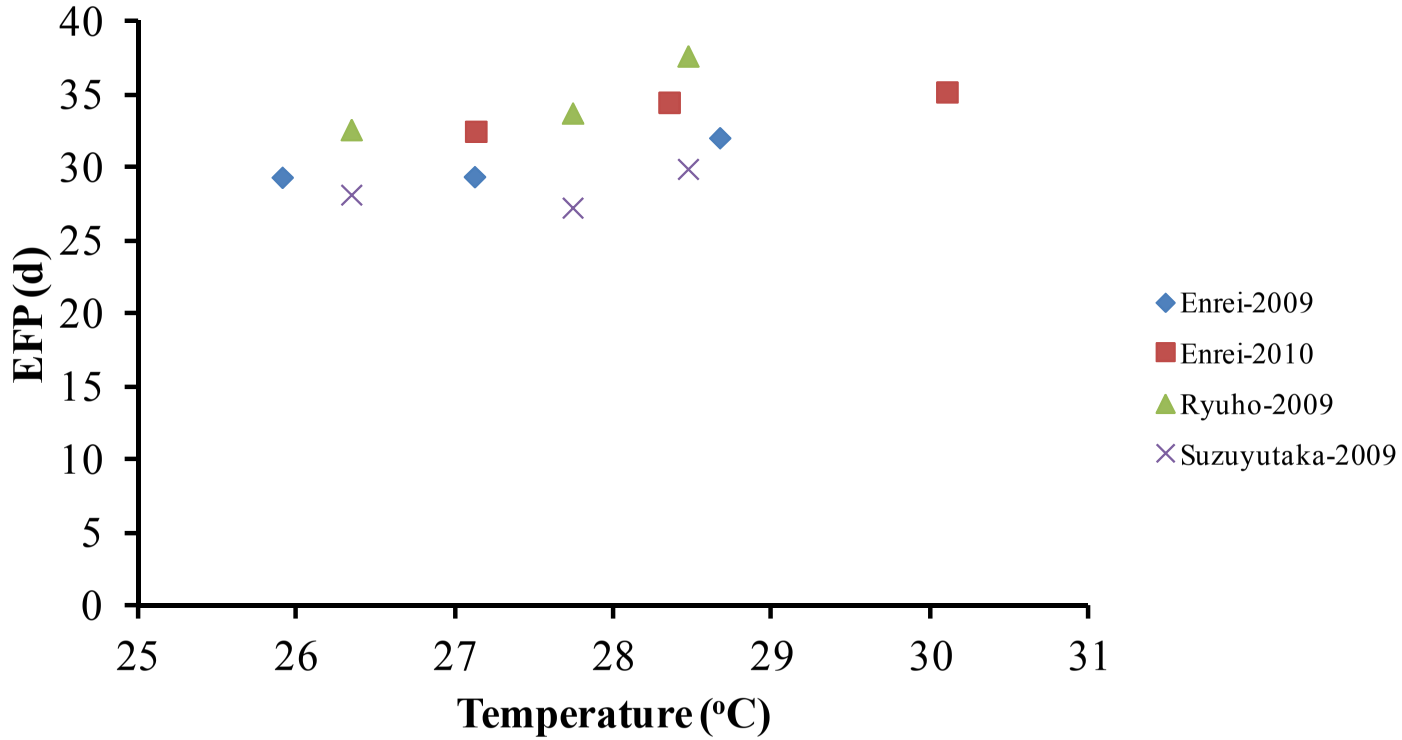

Figure 8. Effective seed filling period as affected by air temperature (for the entire growing season).

Table 5 shows the single correlations between the seed attributes for the Enrei cultivar. The number of cells showed a strong association with the single-seed weight and SGR, yet the cell volume did not show a significant correlation with the other components.

Table 5. Correlation coefficients of seed attributes of soybean cv. Enrei (n=6)

	Single seed weight	SGR	EFP	Cell number
SGR	0.905 *			
EFP	-0.737	-0.953 *		
Cell number	0.963 *	0.968 *	-0.858 *	
Cell volume	-0.730	-0.761	0.732	-0.673

*Significant differences at 5% level of significance

2.4 Discussion

2.4.1 Yield and yield components

A 2–3^{o}C gradient was created most of the time during the growing season, and it partly affected plant phenology. The observed R1 was not affected by increased temperature, except for in 2010 when it was significantly delayed. The temperature during the preflowering period might have largely exceeded the optimum for developmental progress in 2010, the warmest year (data not shown). The R5 stage was consistently delayed by increased temperature in each year. This delay in plant development is a trend different from that shown in previous studies (Craufurd and Wheeler, 2009), which reported enhanced plant development with increased temperatures, including for soybeans (Prasad et al., 2005); however, using a wide range of temperatures, Thomas et al. (2010) found a longer seed-filling period under increased temperatures when they studied the effect of temperature on seed developmental ontogeny, with 32/22°C appearing to be a critical optimum. Therefore, in our study, temperatures above optimum appear to have occurred in 2010 and delayed plant development.

Seed yield was reduced under increased temperatures by 16% to 40% in the current study. The reduction resulting from high temperatures was caused by the reduction of important yield components such as fertile pods, seed number, and seed size, and the poor pod-set is presumably related to delayed onset of seed growth.

Decreased soybean seed yields resulting from temperatures >30^{o}C in controlled-environment chambers have been reported, and were associated with either decreased seed number per plant or decreased single-seed weight (Gibson and Mullen, 1996;

Heinemann et al., 2006); however, biomass production was not considerably affected, even at mean temperatures near 40°C (Allen and Boote, 2000) and the seed yield was not reduced under high-temperature stress induced only at the onset of R5 (Ferris et al., 1999). In contrast, seed yield was reduced under a moderately high temperature of 27.5°C during the entire growing season (Heinemann et al., 2006). In TGC, Ohe et al. (2007) reported a reduction in yield of approximately 30% as a result of a temperature increase of approximately 4°C (27–31°C); Shiraiwa et al. (2006) also found a reduction in yield. In these TGC studies, the detailed mechanisms of reduction were not elucidated. Compared to the above studies, the results obtained in 2009 and 2010 might be unique, because a temperature increase of 3°C resulted in a yield reduction as great as 40% in 2009 and 38% in 2010.

Reviewing these results, one might suspect that plants under high temperatures encountered transient decreases in water content as an artifact of soil moisture control within the first 2 years. This is because irrigation was conducted in only limited times and occurred at the same time in all the plots, boosting soil moisture to field capacity. Plots were then left to lose soil moisture naturally until the next irrigation to resume field capacity; however, in 2011 and 2012, we managed to ensure that the soil was continuously wet enough to prevent any confounding effects of dehydration. The results of the 2011/2012 study showed similar trends in DM accumulation, pod/seed development, HI, and consequent reduction in seed yield resulting from increased temperature.

One possibility, other than drought occurrence, for this large negative effect of high temperature is the reduction in ambient [CO_2] under high temperature, a low light

environment in the TGC, in addition to high VPD, which will be discussed in the next chapter.

The effects of temperature might have been aggravated by the fact that high temperatures occurred from flowering to early seed filling. It is generally recognized that the potential impacts of climate change and its resultant temperature change on crop production are particularly evident when high temperatures are induced during the postanthesis phase in soybeans (Hatfield et al., 2011). In the common bean, another crop legume, heat stress from near anthesis to R8 was found to affect the pollen viability and yield of sensitive genotypes (Porch and Jahn, 2001). However, if temperature increase had direct effect on yield through reduction of pollen viability and pod set ratio is a matter for further studies.

2.4.2 Single seed weight and growth attributes

The increased temperature decreased the final single-seed weight, which has also been reported in previous studies (Mochizuki et al., 2005; Gibson and Mullen, 1996; Thomas et al., 2010). Although no attempt was made to quantify them, it was apparent that the number of shriveled seeds (Fig. 3) was higher at Ta+3 than at the near ambient temperature and may be associated with the final seed size (Thomas et al., 2010).

Considering its formation process, the seed size in legumes is dependent on the individual seed growth rate and the duration of seed filling (Munier-Jolain and Ney, 1998; Egli, 2004; Sadras and Egli, 2008). The seed-filling period was significantly increased by the increased temperatures in this study and the increase was consistent in all three cultivars. Longer seed-filling period would be expected to result in large final

seed size. However, the slower rate of accumulation of dry matter in the seed (lower SGR) under the warmer treatment might have affected the final single-seed weight. In fact, the calculated seed filling period (EFP) was negatively correlated with both individual single seed weight and SGR (Table 5).

The number of cells per cotyledon showed a similar trend as the SGR, a result that is in agreement with the commonly reported relationship between the two parameters (Munier-Jolain, 2008). Thus, the reduction in the SGR with the increased temperature is attributable to a reduction in the cell number, with the temperature condition during the period of cell division being of particular importance. Because major cell division in the cotyledons is complete by approximately 15 to 20 d after flowering (Kato et al., 1954), the higher temperatures in the period between R1 and R5 in 2010 might have resulted in fewer cells, lower SGRs and smaller seed sizes than in 2009. Thomas et al. (2010) also found that when temperatures are relatively high, the higher the temperature the slower the SGR and the negative effects of elevated temperature appeared to have occurred early in the reproductive cycle. This interpretation is also supported by Ferris et al. (1999) who exposed pot-grown soybean plants to a high temperature stress of 30 °C, with and without water deficit, for eight days starting at R5. Although no reduction in individual size was found in either the well-watered or stressed pots, significant reductions in the seed number under the high-temperature and water-stressed conditions were observed.

Another important determinant of seed size is the cell volume, which, in this study, decreased with the increased temperature in 2009, whereas the opposite trend occurred in 2010. The inconsistency in the results between the two years might have been caused by some experimental error that likely occurred in 2010. Previous studies found

that warmer temperatures reduced the soybean cell size (Thomas et al., 2010) or the cell volume in the common bean (Sexton et al., 1997). Nevertheless, the strong correlations between the cotyledon cell number and SGR and between the cell number and final seed size (Table 5) might explain the mechanisms by which the seed size was reduced by the increased temperature in the present study.

Chapter 3

Effects of increased temperature on dry matter production

3.1 Introduction

The soybean responds to environment not only through seed number and seed size but also through dry matter production. The dry matter response to increased temperature has rarely been evaluated because it should be done on crop basis rather than plant basis, which is difficult. Both intercepted radiation and radiation use efficiency can be affected by temperature (Laffitte and Edmeades, 1997), resulting in reduced dry matter production.

Because of the growing concern about global warming, researches have been conducted over the years in controlled environment in many crops and on plant basis but the results are not consistent. Increasing mean air temperature up to 32.5^{o}C resulted in decreased total biomass in soybean (Baker et al., 1989). Similarly, plants grown under high mean air temperature up to 34^{o}C from 10 days after sowing (DAS) to maturity had less total biomass than the control (Koti et al., 2007). However, biomass was not considerably affected by increased temperature of up to 39^{o}C (Allen and Boote, 2000) and HI was reported to be decreased by increasing mean air temperature beyond 26^{o}C (Boote et al., 2005).

The objective of this study was to investigate the effects of increased temperature on dry matter production of soybean using temperature gradient chamber.

3.2 Materials and Methods

3.2.1 Materials and environmental description

Soybean cultivar Enrei, determinate and of maturity group IV, was grown under various temperatures in TGCs, as described in chapter 2. Three temperature treatments, near ambient temperature (Ta), ambient temperature + 1°C (Ta + 1), and ambient temperature + 3°C, in 2009 and 2010, and ambient temperature + 2°C, in 2011 and 2012 (Ta + 2/Ta + 3), were established by dividing the rows along which the temperature gradient was created.

3.2.2 Measurements

(1) DM accumulation

The aboveground parts of six plants per treatment were sampled at the onset of flowering (R1), the beginning of seed filling (R5) (2011 and 2012), at 5 days after R5 at Ta (in 2009), or at 10 days after R5 at Ta (in 2010) and eight plants at harvest maturity (R8) to determine the changes in aboveground DM accumulation. At harvest maturity, plant components were separated into leaves (including petioles), stems, pod shells, and seeds that were used to calculate the final single-seed size, seed number, seed yield, and total aboveground DM.

(2) Leaf photosynthetic rates and stomatal conductance

In 2011 and 2012, photosynthetic rate (Pn) and stomatal conductance (gs) were measured in the central leaflets of fully developed leaves of three plants per treatment (Ta and Ta + 2) once a week from near flowering, using LI-6400 (LI-COR, Inc., Lincoln, Nebraska, USA). According to these measurements, which were conducted during the day, CO2 concentration [CO2] at Ta + 2 was 350 ± 13 μL/L compared to 372 ± 8 μL/L under Ta.

(3) Carbon isotope discrimination

The carbon isotope discrimination of milled samples (0.5 mg for seed and 2.0 mg for leaf) collected at harvest maturity from Ta and Ta + 2 plots was determined by mass spectrometry (Delta V; Thermo Fisher Scientific) at Kyoto University Ecological Centre. Carbon isotopic composition of seed and leaf samples was expressed relative to the standard Pee Dee Formation of Belemnite.

Carbon isotope compositions of seeds ($\delta^{13}C$) were converted to carbon isotope discrimination ($\Delta^{13}C$) using the formula by Farquhar et al. (1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}$$

$\delta^{13}C_{atm}$: Carbon isotope composition of air (-8 ‰)

$\delta^{13}C_{plant}$: Carbon isotope composition of seeds (measured values)

$\Delta^{13}C$ were multiplied by 1000.

3.2.3 Statistical analysis

The effects of increased air temperature on seed yield, yield components, DM accumulation, and carbon isotope discrimination (CID) were evaluated using an analysis of variance (ANOVA) with statistical software SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and Cropstat.

3.3 Results

3.3.1 Temperature environment and vapor pressure deficit

The day, night, and mean air temperatures for the entire growing season and the estimated vapor pressure deficit at different developmental stages in four years experiment are common with those presented in Table 1 in chapter 2.

3.3.2 DM accumulation

The total DM at R8 was reduced by 27% in 2009, 9% in 2010, 26% in 2011, and 11% in 2012 (Table 6). The effect of increased temperature on DM accumulation was observed during most of the growing period and a major part of the differences occurred in the period from 35 to 65 days after sowing (DAS) in 2009, 32 to 55 DAS in 2010, 32 to 50 DAS in 2011, and 29 to 56 DAS in 2012. There were no evident differences in plant responses between Ta and Ta + 1, but there were differences between Ta and Ta + 2/Ta + 3, although they were significant only in 2010 and 2011 (Fig. 9). The ANOVA also showed that the differences in accumulated DM between Ta and T + 2/Ta + 3 at R8 were

significant only in 2011. Because of the effects of temperature on crop phenology, the developmental stages at the time of plant sampling differed among treatments. For example, when sampled at 65 DAS in 2009, the plants under Ta were at 5 days after R5, whereas the plants under Ta + 3 were harvested at R5.

Increasing temperature also significantly affected the fraction of total aboveground DM allocated to the seed HI (Table 6). HI was reduced by approximately 18% in 2009, 32% in 2010, 6% in 2011, and 5% in 2012. As shown in Fig. 6, chapter 2, the progress of pod development was delayed under warmer conditions and it resulted in reduced HI. In Ta + 3, DM of the leaf + petiole was significantly greater than under Ta, indicating leaf senescence was late in the warmer treatment (Table 6).

Table 6. Response of DM production to increased temperature

Year and Treatment	Air temperature[1)]			DM at R8			HI
	Mean	Day	Night	Leaf + petiole	Stem + podshell+ seed	Total	
	°C	°C	°C	(g m^{-2})	(g m^{-2})	(g m^{-2})	
2009							
Ta[2)]	25.9	28.2	23.7	49	674	722	0.56
Ta + 1	27.1	29.3	25.0	109	545	654	0.47
Ta + 3	28.7	31.1	26.2	93	432	525	0.46
2010							
Ta	27.1	29.5	24.7	39	579	617	0.53
Ta + 1	28.3	30.7	26.0	74	489	563	0.49
Ta + 3	30.1	32.6	27.6	148	412	560	0.36
2011							
Ta	25.7	27.9	23.4	58	806	864	0.56
Ta + 1	26.6	29.0	24.2	138	742	880	0.48
Ta + 2	27.4	29.7	25.1	67	573	640	0.53
2012							
Ta	26.9	29.3	24.6	152	745	897	0.48
Ta + 1	27.5	30.0	25.0	152	705	857	0.47
Ta + 2	28.8	31.5	26.0	154	644	798	0.46
ANCOVA							
Temperature (T)				5.33 (<0.05)	88.18 (<0.01)	14.29 (<0.01)	22.10 (<0.01)
Year (Y)				0.98 (n.s.)	3.86 (<0.05)	2.17 (n.s.)	1.49 (n.s.)
T x Y				0.87 (n.s.)	3.88 (<0.05)	2.06 (n.s.)	1.44 (n.s.)

[1)] Temperature for the entire growing season, [2)] Near ambient temperature

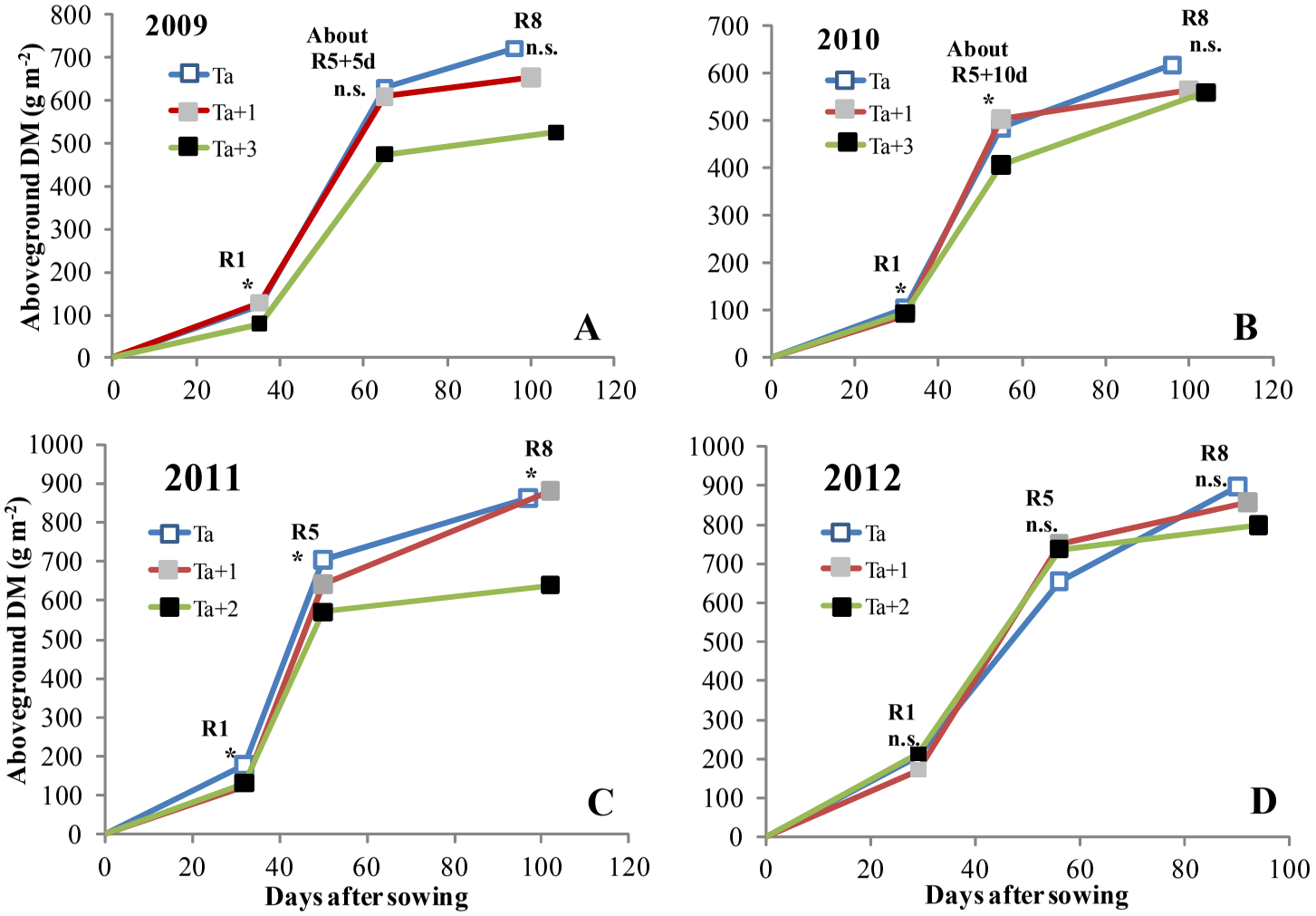

Figure 9. Temporal changes in aboveground dry matter (DM) of soybean cv. Enrei in 2009 (A), 2010 (B), 2011 (C), and 2012 (D). The statistical significances are comparisons between Ta and Ta + 2/Ta + 3 of each year.

3.3.3 Photosynthesis and stomatal conductance

Increasing the temperature by approximately 2^{o}C reduced the photosynthetic rates, with 9–16% lower values under Ta + 2 than under Ta (Fig. 10A). Two of the four observed reductions in photosynthetic rates appeared to be associated with the reduction of stomatal conductance (Fig. 10B).

3.3.4. Carbon isotope discrimination

The CID of seed samples ranged from 19.50 to 20.39‰ in 2011 and from 20.06 to 20.39‰ in 2012, with the values being significantly lower under warmer treatments; however, there was a significant effect of temperature on CID in leaf samples only at 10% level (Table 7).

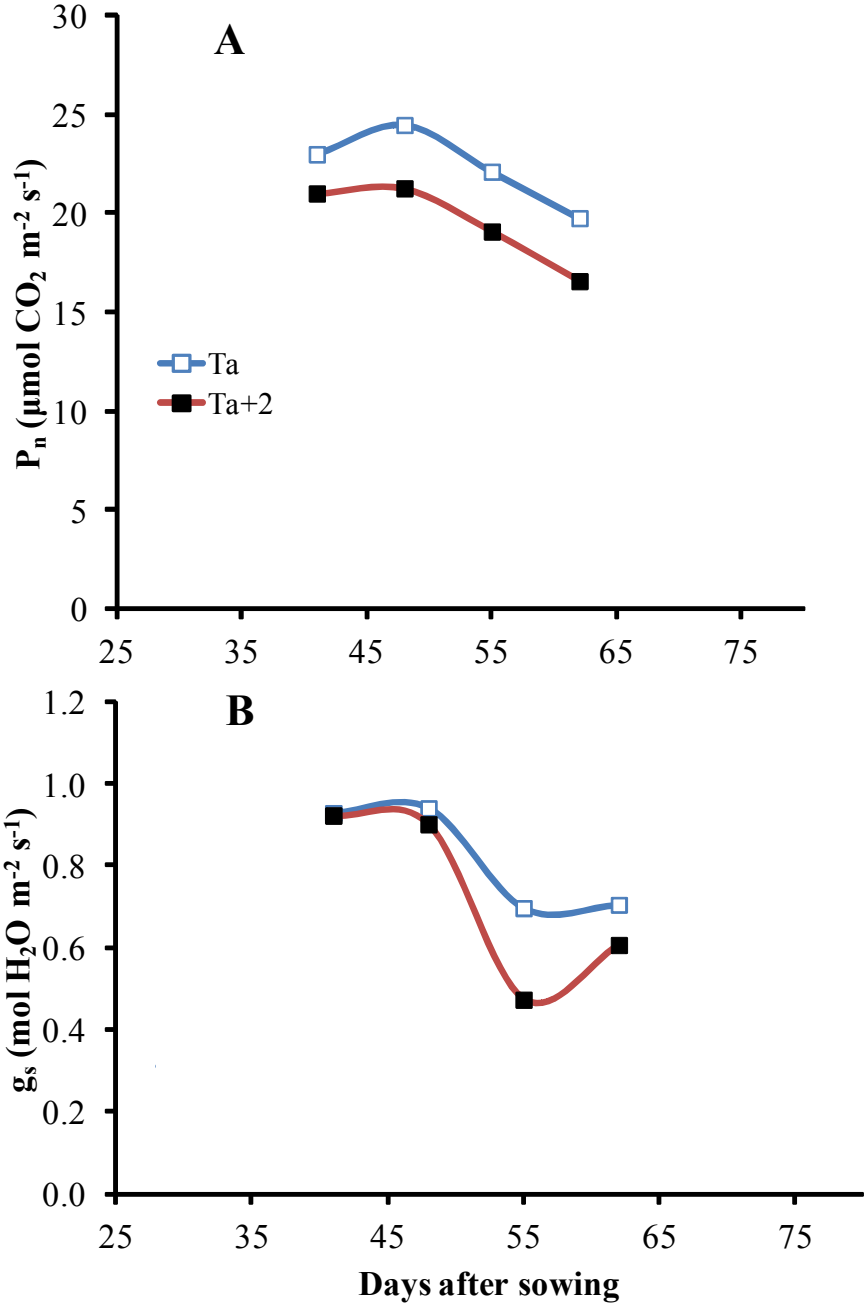

Figure 10. The response of photosynthesis (A) and stomatal conductance (B) to increased temperature in 2011.

Table 7. The response of carbon isotope discrimination (CID) to increased temperature in 2011 and 2012

Year and Treatment	CID	
	Seed (‰)	Leaf (‰)
2011		
Ta	20.39	21.77
Ta+2	19.50	20.84
2012		
Ta	20.39	21.52
Ta+2	20.06	21.42
ANOVA	F value (P)	
Treatment (T)	12.09 (<0.05)	5.71 (<0.1)
Year (Y)	3.04 (n.s.)	4.27 (n.s.)
T ×Y	3.54 (n.s.)	4.63 (<0.1)

3.4 Discussion

A 2–3^{o}C increase in temperature resulted in reduced dry matter production and lower HI, and seed yield discussed in previous chapter. The responses of TDM and seed yield were unexpectedly greater than from the earlier studies. The reduction of TDM was associated with the decline of photosynthesis and stomatal conductance.

One possibility for this large negative effect of high temperature is the reduction in ambient [CO_2] under high temperature and a low light environment in the TGC. Because of the nature of TGCs, where air flow was created from the entrance (near Ta) to Ta + 2 or Ta + 3 along the rows of growing plants, there was a reduction in ambient [CO_2], despite the relatively high air flow rate inside the chamber. Thus, the substantial yield reduction with an increase in temperature might be enhanced because these plants were potentially exposed to lower-than-ambient [CO_2] and a 20% reduction in incident radiation through the film in the tunnel. However, the difference in [CO_2] of 20 μL/L mentioned earlier does not appear to contribute in major part to the observed change in photosynthetic rate and DM accumulation.

In addition, high VPD resulting from increased temperatures might have contributed to reduced photosynthesis through stomatal closure part of the time (Table 1; Fig. 11). There were days between anthesis and R5 when maximum temperatures were as high as 35°C in 2009 and 36°C in 2010, and there existed a concomitant increase in VPD up to 2.5 or even 3.0 kPa (Fig. 11). As has been demonstrated (Bunce, 1984; Fletcher et al., 2007), the increase in VPD might result in stomatal closure to a greater extent in the warmer treatment than in the cooler treatment. Lower stomatal conductance was observed

in plants grown under high temperature (Fig. 10B). Although it has not been examined for soybean cultivar Enrei, some commercial cultivars tend to restrict transpiration with increasing VPD >2.1 kPa (Fletcher et al., 2007). Bunce (1984) found that stomatal conductance at a VPD of 3 kPa was lower than that at 1 kPa, and a 50% reduction in stomatal conductance resulted in a 20% reduction in photosynthesis. In this study also, high VPD in high-temperature plots might have affected the photosynthetic rate and yield formation process.

In general, a reduction in Pn is caused not only by reduced g_s but also by reduced mesophyll activity. Considering the coordinating behavior of g_s and mesophyll activity in leaf photosynthesis (Wong et al., 1979), it is difficult to point out g_s as a cause of varied Pn, simply based on the association of the two variables; however, the analysis of CID, a measure of balance between CO_2 fixation in the mesophyll and CO_2 diffusion from the atmosphere into intercellular spaces (Farquhar et al., 1982), showed a significant reduction after high-temperature treatment (Table 7). A reduction in CID with increased temperature partially meets with above-mentioned decline in g_s in the high-temperature treatment, and suggests that reduction in g_s, rather than mesophyll activity, was most likely more responsible for the reduction in DM productivity.

Temporal changes in aboveground DM (Fig. 10) showed that DM accumulation was more affected by the treatments from R1 to R5 than in other periods. Considering that temperatures were highest from R1 to R5 during the growing season (Fig. 5), this also shows that DM accumulation was limited under high temperatures, which might be associated with restricted stomatal conductance.

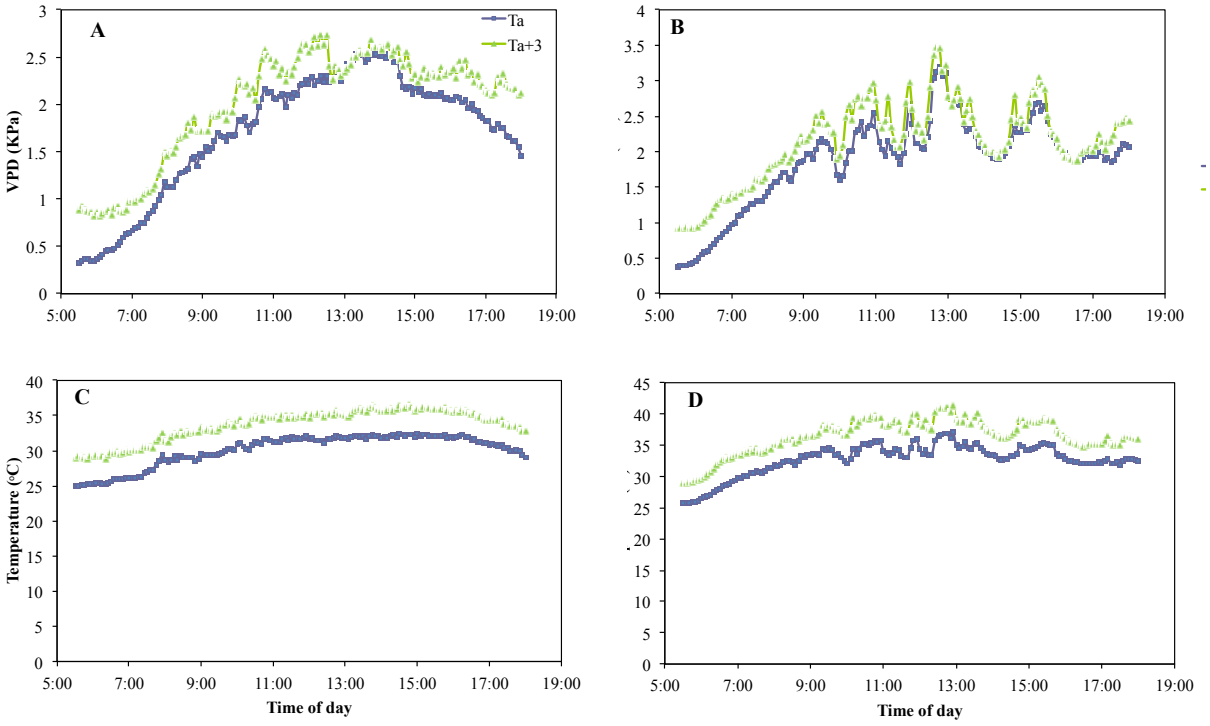

Figure 11. Vapor pressure deficit (VPD) and temperature on August 14, 2009 (A and C, respectively), and August 17, 2010 (B and D, respectively).

In terms of HI, this study confirmed previous findings that temperatures increased to above the optimum decreases DM partitioning to seed in many crops, including soybeans (Boote et al., 2005; Shiraiwa et al., 2006). Indeed, combined data from the four study years show that HI, seed yield, and total DM are consistently reduced by increased temperatures (Fig. 18).

The reduction in HI was associated with a reduced number of pods/seeds and smaller seeds. Because seed size reduction is considered to be the result of reduced cell number, which is determined by mid-seed filling (R5–R6) (chapter 2), these processes related to HI reduction are assumed to be a consequence of internal and external factors from after R1 until R5. However, whether the poor pod set and seed development is a result of reduced DM production or a direct effect of temperature remains to be elucidated.

A particular feature of these TGC experiments is that high temperatures caused delayed leaf senescence (or green stem disorder), and this phenomenon was more marked in 2010 than in 2009 (Table 6). In 2010, more green leaves were harvested at maturity and might have compensated the final aboveground DM as compared to 2009, when leaves were old and many had abscised by harvest time.

Considering that soybeans induce senescence primarily to fill their seedpods (Sinclair and de Wit, 1976; Egli, 2004), the delayed pod and seed growth and smaller seeds in the higher temperature treatment might have caused delayed senescence resulting from sink limitation. A temperature of a few degrees higher also caused delayed senescence in some soybean cultivars, and the seeds were smaller in an experiment conducted in the same TGC; however, the magnitude of the delayed senescence resulting

from increased temperatures was cultivar dependent, with some cultivars not showing any delayed senescence (Mochizuki et al., 2005). This fact and the results of this study suggest a need to explore genetic variation in the responses of soybean performance to increased temperature.

3.5 Conclusion

Large effects of an increase in temperature by a few degrees were observed in our TGC studies conducted under field-like conditions at Kyoto, a warm region of Japan. The yield was reduced in plants treated with an increase in temperature through reductions of pod and seed numbers associated with a decline in DM production. It was suggested that the reduction in ambient [CO_2] and light intensity and the concomitant increase of VPD with increased temperature in the TGC exacerbated the effects of increased temperature. The temperature-related reduction in yield is likely to occur in warm regions, where high temperatures coincide with atmosphere dryness and it might occur more often with the climate change; however, whether the dryness of the atmosphere exacerbates the effects of high temperature through drought is a matter for further studies.

Chapter 4

Interactive effects of high temperature and drought

4.1 Introduction

Although the mechanism has not been fully understood, evidences have increased for potential reduction of seed yield under increased temperature in soybean grown in the warm regions (Egli and Wardlaw, 1980; Shiraiwa et al., 2006; Ohe et al., 2007). Increasing temperature above the optimum could have a direct impact on pod and seed number (Egli and Wardlow, 1980; Mochizuki et al., 2005) and seed size (Gibson and Mullen, 1996; Zheng et al., 2000; Mochizuki et al., 2005; Thomas et al., 2010), resulting in reduction of harvest index (HI). In addition biomass production also appears to be affected as mentioned later.

There are increasing suggestions that temperature-related reductions of plant biomass may be aggravated by water deficit (Shan and Paulsen, 2003; Mittler, 2006; Prasad and Staggenborg, 2008; Prasad et al., 2011). On one hand, evaporative demand increases with temperature, even if relative humidity remains constant, causing the stomata to close, which reduces transpiration and photosynthesis and increases leaf temperature further because of decreased latent heat loss (Long and Port, 2010). On the other hand, it is well known that plants tend to close stomata and reduce transpiration in

water deficit environments (Hsiao, 1973), which in turn increases plant temperature. The stomata closure and reduced stomatal conductance can negatively affect photosynthetic rates and depress crop yield (Danneberger, 2000; Gilbert et al., 2011). Thus high temperature interacts with drought exacerbating the impacts of stress.

However, information on interactive effects of drought and heat stress is limited (Prasad and Staggenborg, 2008) and the combined response can not be estimated from the response of plants to each stress applied individually (Mittler, 2006). Furthermore, the response has rarely been examined under field condition or near-field conditions.

Therefore, the objective of this study was to examine the interaction of increased temperature and moisture deficit on dry matter production and yield of soybean grown under near-field conditions at warm region of Japan.

4.2 Materials and Methods

4.2.1 Material and environmental description

This experiment was conducted in the same environment described in the previous chapters as a part of another study on the response of soybean yield and yield components and biomass production to increased temperature.

In 2011 and 2012, soybean cv. Enrei, maturity group IV, was grown under various temperatures treatments in TGCs. Plants were sown on July 12 in both years in the soil culture bed in four TGCs, arranged into four rows of 0.25 m in width with a 22 m length and a 0.25 m intra-row spacing. Three temperature treatments, Ta (near ambient

temperature), Ta+1 (ambient temperature +1 °C) and Ta+2 (ambient temperature +2 °C), were established by dividing the rows along which the temperature gradient was created. The TGC was equipped with an irrigation system containing an irrigation pipe located 50 cm below the soil surface. Water was supplied through the pipe to raise the water table to approximately 40 cm below the soil surface, and the water was evenly distributed throughout the entire soil culture bed. At approximately flowering time, drought treatment was started by withholding water supply in two out of four TGCs and the other two served as control. Soil moisture content was monitored with the time domain reflectometry (TDR) meter (SONY Tektronix Co. Ltd., Japan) installed vertically to a depth of 30 cm from the surface to give an average soil moisture content of the 30 cm depth layer. The volumetric water content was on average 24% throughout the entire growth period in the control while it was left to drop down to around 15% (Fig. 13).

4.2.2 Measurements

The developmental stages were determined according to Fehr and Caviness (1977). In 2012 only, the above ground parts of six plants per treatment were sampled at R1, at R5 of Ta. At R8 in both 2011 and 2012, about eight plants were harvested and plant components were separated into leaves (including petioles), stems, pod shells and seeds that were used to calculate the final single seed weight, seed number, seed yield and total aboveground dry matter. Leaf photosynthesis and stomatal conductance were measured in the central leaflets of fully developed leaves of three plants per treatment (Ta and Ta+2^{o}C) with LI-6400 (LI-COR, Inc., Lincoln, NE, USA) once a week from near

flowering. The carbon isotope discrimination of milled samples (0.5mg for seed and 2.0mg for leaf) collected at harvest maturity from all plots was determined by mass spectrometry (Delta V, Thermo fisher Scientific) at Kyoto University Ecological Centre. Carbon isotopic composition of seed and leaf samples was expressed relative to the standard Pee Dee Formation of Belemnite. Data from 2011 and 2012 were combined for the analysis of waters vs. temperature interactions.

4.2.3 Statistical analysis

The effects of increased air temperature and drought stress on yield components, dry matter accumulation and CID were evaluated using an analysis of variance with CropStat. The statistical significance was assessed with F-test.

4.3 Results

The data in Tables 8 and 9 and Fig. 12 regarding control treatment are common with those presented in the previous chapter in which the effect of temperature was specifically addressed. But hereafter the description for the temperature is repeated in order to facilitate the examination of interactive effects of temperature and water treatments.

4.3.1 Temperature environment, vapor pressure deficit and soil moisture

The day, night and mean air temperatures for the entire growing season are shown in Table 12. In the TGC, the lower temperature treatment (Ta) was near the ambient temperature, and a temperature difference of approximately 2 °C was achieved in both years for the day, night and mean temperatures. Changes in the daily mean temperature during the entire growing season are shown in Fig. 12. A temperature gradient of approximately 2 °C was observed most of the time, and the indoor temperature tended to track the diurnal fluctuation of the outdoor temperature. In general, plants experienced a period of relatively high temperature (over 30°C) prior to the beginning of seed filling, with some fluctuations, especially in 2011. The temperatures dropped in the mid-seed filling and then fluctuated until the plants reached maturity.

Estimated day, night and mean vapor pressure deficit (VPD) from emergence (VE) to R5 and during the seed filling period (R5-R7) are presented in Table 8. The daily mean VPD ranged from 0.9 to 1.6kPa and the day average reached around 2.1 kPa during the period before the onset of flowering. The VPD tended to be higher in the warmer treatment as compared to near ambient temperature and the trend was similar in both well-watered and drought environments. There was no significant interaction of water and temperature on VPD.

Soil moisture content in control and drought conditions are shown in Fig. 13. Before drought was imposed soil moisture was high in both years, oscillating between 20 and 25%. Few days after withholding water supply, moisture content dropped to around 15% in the drought conditions. Averaged values of soil moisture content for the entire growing season under control were about 24% in both years.

Table 8. Estimated vapor pressure deficit during different stages in the TGC, in 2011 and 2012

Year	Treatment		VPD (kPa)									
	Water	Air temperature	VE-R1			R1-R5			R5-R7			
		Mean	Day	Night	Mean	Day	Night	Mean	Day	Night	Mean	Day	Night
2011	Control	Ta[1)]	25.7	27.9	23.4	1.17	1.67	0.68	0.92	1.26	0.58	0.93	1.30	0.56
		Ta+1	26.6	29.0	24.2	1.31	1.82	0.79	0.95	1.23	0.67	0.97	1.35	0.59
		Ta+2	27.4	29.8	25.1	1.35	1.73	0.96	1.21	1.44	0.97	1.21	1.58	0.84
	Drought	Ta	26.3	28.3	24.3	1.12	1.58	0.65	1.00	1.39	0.61	0.90	1.29	0.51
		Ta+1	26.8	29.1	24.6	1.26	1.76	0.77	1.14	1.51	0.76	1.03	1.52	0.55
		Ta+2	28.1	30.2	26.0	1.34	1.88	0.81	1.26	1.63	0.89	1.20	1.71	0.69
2012	Control	Ta	26.9	29.3	24.6	1.25	1.71	0.79	1.05	1.41	0.69	0.81	1.16	0.45
		Ta+1	27.5	30.0	25.0	1.35	1.83	0.86	1.08	1.43	0.73	0.83	1.21	0.46
		Ta+2	28.8	31.5	26.0	1.53	2.06	1.00	1.51	1.89	1.14	1.23	1.62	0.84
	Drought	Ta	27.0	29.3	24.7	1.24	1.70	0.78	1.15	1.55	0.75	0.88	1.29	0.47
		Ta+1	27.3	29.8	24.8	1.36	1.83	0.88	1.22	1.62	0.82	0.92	1.33	0.51
		Ta+2	28.6	31.4	25.9	1.55	2.07	1.03	1.58	1.99	1.17	1.34	1.80	0.88
ANOVA			F value (P)									
Water (W)			0.63	0.00	1.50	11.75	15.87	2.63	1.17	5.36	0.23	
			(n.s.)	(n.s.)	(n.s.)	(<0.01)	(<0.01)	(n.s.)	(n.s.)	(<0.05)	(n.s.)	
Temperature (T)			70.9	21.61	34.95	52.42	24.11	96.30	24.87	24.38	22.86	
			(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	
Year (Y)			50.9	14.57	26.91	37.05	30.17	33.09	0.70	1.11	0.28	
			(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(n.s.)	(n.s.)	(n.s.)	
WxT			0.52	1.42	0.68	1.08	0.62	2.03	0.12	0.37	0.18	
			(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	

[1)] Near ambient temperature

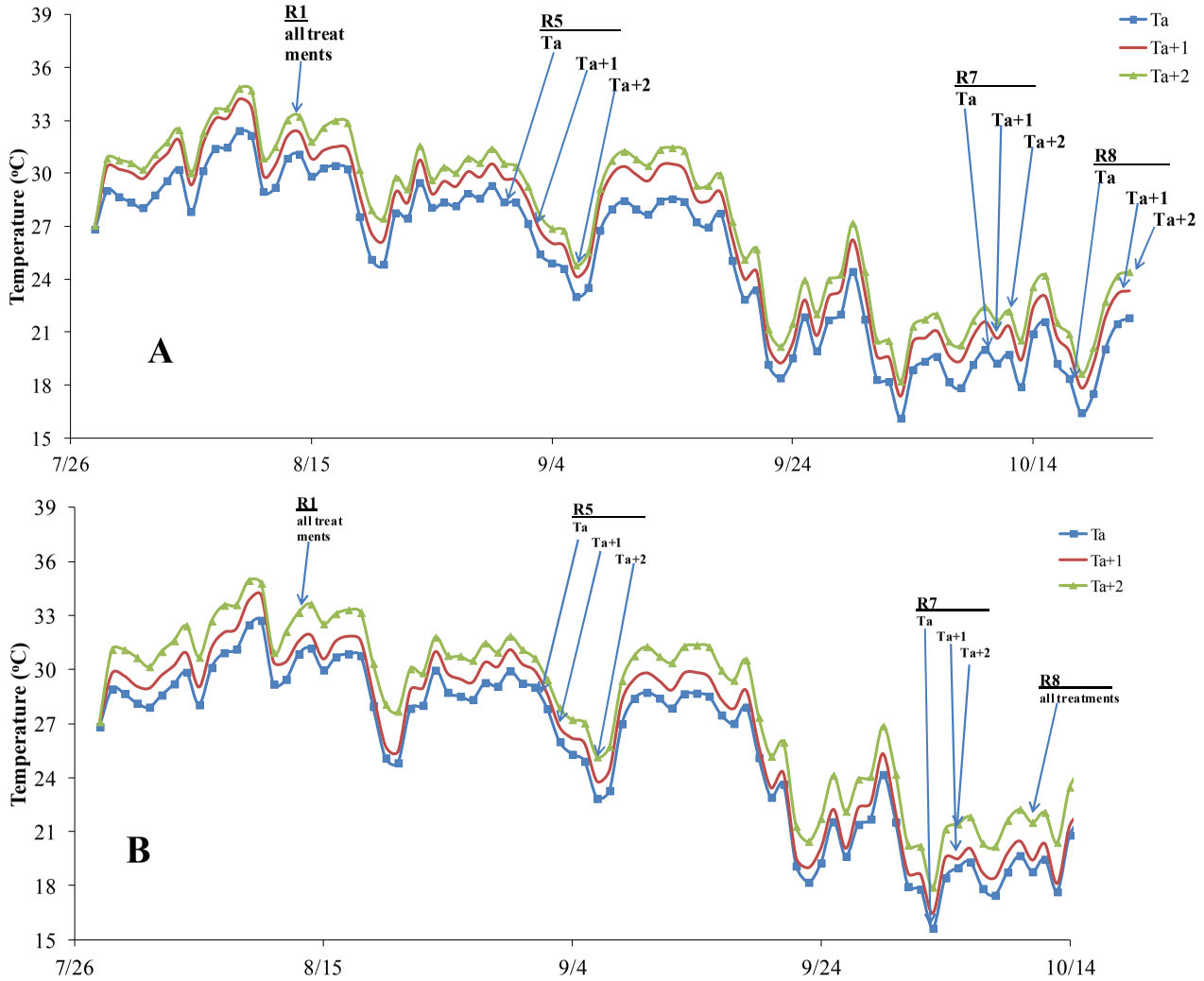

Figure 12. Daily changes in mean air temperature at different treatments: control (A) and drought (B) in TGC in 2011.

4.3.2 Developmental stages

Temperature increase by 2^{o}C or drought did not significantly affect the onset of flowering (R1) (Table 9). However, the beginning of seed filling (R5) was significantly delayed by increased temperature. In the plants grown under increased temperature, R5 occurred 5 and 4 days later, in the control and drought-induced plots, respectively and water treatment did not have significant effects on the days from R1 to R5. The duration of seed filling (R5-R7) was significantly shorter under drought conditions compared with well-watered conditions, irrespective of temperature treatment. Temperature and water did not have significant interaction on the duration of the developmental stages.

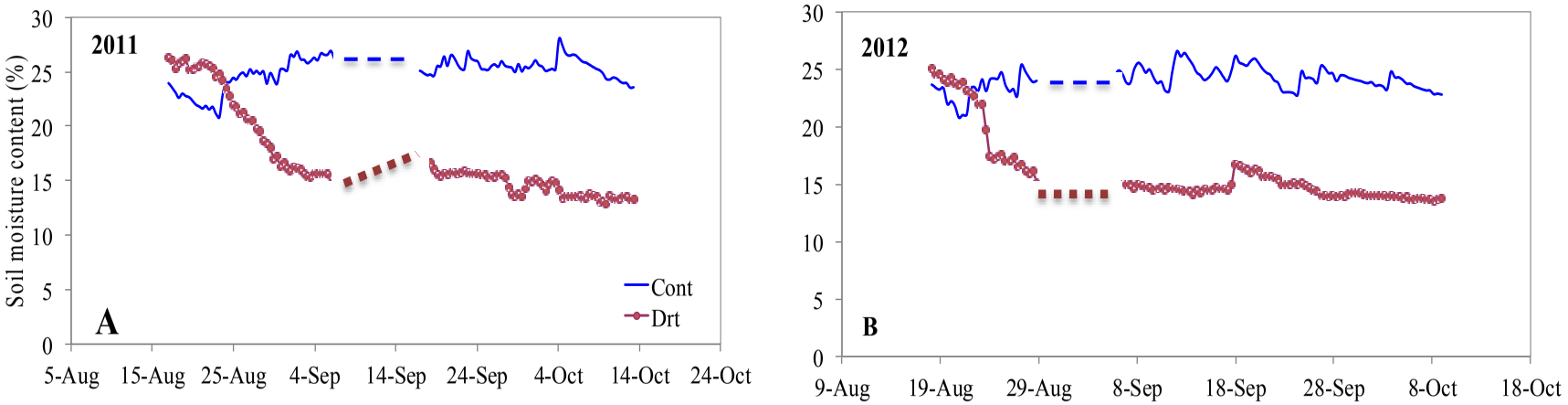

Figure 13. Soil moisture content at two water treatments, control and drought, in 2011 (A) and 2012 (B).

Table 9. Temperature response of phenology and yield components of soybean cv. Enrei.

Year	Treatment		Days from		
	Water	Temperature	Emergence to R (d)	R1 to R5 (d)	R5 to R7 (d)
2011	Control	Ta	27	19	40
		Ta+1	27	22	40
		Ta+2	27	24	38
	Drought	Ta	26	20	32
		Ta+1	27	22	33
		Ta+2	27	24	29
2012	Control	Ta	25	19	37
		Ta+1	25	20	38
		Ta+2	25	23	39
	Drought	Ta	25	20	30
		Ta+1	25	22	30
		Ta+2	25	24	28
ANOVA			F value (P)		
Water (W)			5.52 (<0.05)	2.38 (n.s.)	79.15 (<0.001)
Temperature (T)			0.88 (ns)	14.40 (<0.001)	1.36 (ns)
Year (Y)			79.7 (<0.001)	0.07 (n.s.)	2.92 (n.s.)
WxT			0.88 (n.s.)	0.05 (n.s.)	0.65 (n.s.)

4.3.3 Yield components and dry matter accumulation

Seed yield and yield components of soybean grown under different water regimes and that subjected to increased temperature are presented in Table 10. Plants grown under increased temperature had significantly fewer pods, fewer seeds and smaller seeds than the plants grown under near ambient temperature similar to that reported in chapter 2. On average, water-stressed plants produced 18-30% fewer pods, 15-30% fewer seeds (on area basis) and 11 to 15% smaller seeds as compared to well-watered plants. Seed yield was significantly affected by increased temperature regardless of water regime, being reduced by 30 and 27% in 2011, and 16 and 30% in 2012, under well-watered conditions and drought, respectively. However, the interaction of water and temperature treatments on any of yield and yield components was not significant, although the P value for yield was about 0.2. Thus combination of temperature and water did not synergistically affect the way with which seed yield or yield components responded to these environmental factors.

Increased temperature significantly reduced the TDM at harvest maturity, irrespective of water regime, with the reduction ranging from 11 to 26% (Table 10, Fig. 14). The increase of dry matter after R1 was clearly lower under drought conditions than under well-watered conditions (Fig. 14B). However, the difference in dry matter accumulation from R1 to R5 was not significant in 2012 (Fig. 14A). The analysis of variance showed that there was no significant interaction of water and temperature on DM production (Table 10), although the P value water by temperature interaction is about 0.15.

Table 10. Temperature responses of and yield components and DM of soybean cv. Enrei.

Year	Treatment[1)]		Pod no.	Seed no.	Single seed weight[3)]	Seed yield	Total DM	HI
	Water	Air temperature	(m^{-2})	(m^{-2})	(mg)	(m^{-2})	(m^{-2})	
2011	Control	Ta[2)]	983	1844	262	483	864	0.56
		Ta+1	1005	1717	242	416	880	0.48
		Ta+2	893	1442	233	336	640	0.53
	Drought	Ta	812	1452	224	324	558	0.58
		Ta+1	739	1254	213	269	575	0.47
		Ta+2	656	1499	199	295	575	0.51
2012	Control	Ta	910	1641	248	435	897	0.48
		Ta+1	895	1589	240	407	857	0.47
		Ta+2	818	1458	235	365	798	0.46
	Drought	Ta	734	1331	230	327	680	0.48
		Ta+1	583	1065	214	278	626	0.44
		Ta+2	500	961	196	239	563	0.42
ANOVA								
Water (W)			45.61 (<0.001)	20.26 (<0.001)	74.42 (<0.001)	84.21 (<0.001)	74.44 (<0.001)	0.68 (n.s.)
Temperature (T)			5.21 (<0.05)	2.92 (<0.1)	17.04 (<0.001)	14.07 (<0.001)	6.34 (<0.01)	5.65 (<0.05)
Year (Y)			8.77 (<0.01)	6.05 (<0.05)	0.26 (n.s.)	0.87 (n.s.)	4.42 (<0.05)	15.23 (<0.001)
WxT			1.01 (n.s.)	1.00 (n.s.)	0.64 (n.s.)	1.83 (n.s.)	2.15 (n.s.)	0.50 (n.s.)

[1)] Temperature for the entire growing season, [2)] Based on dry matter, [3)] Near ambient temperature

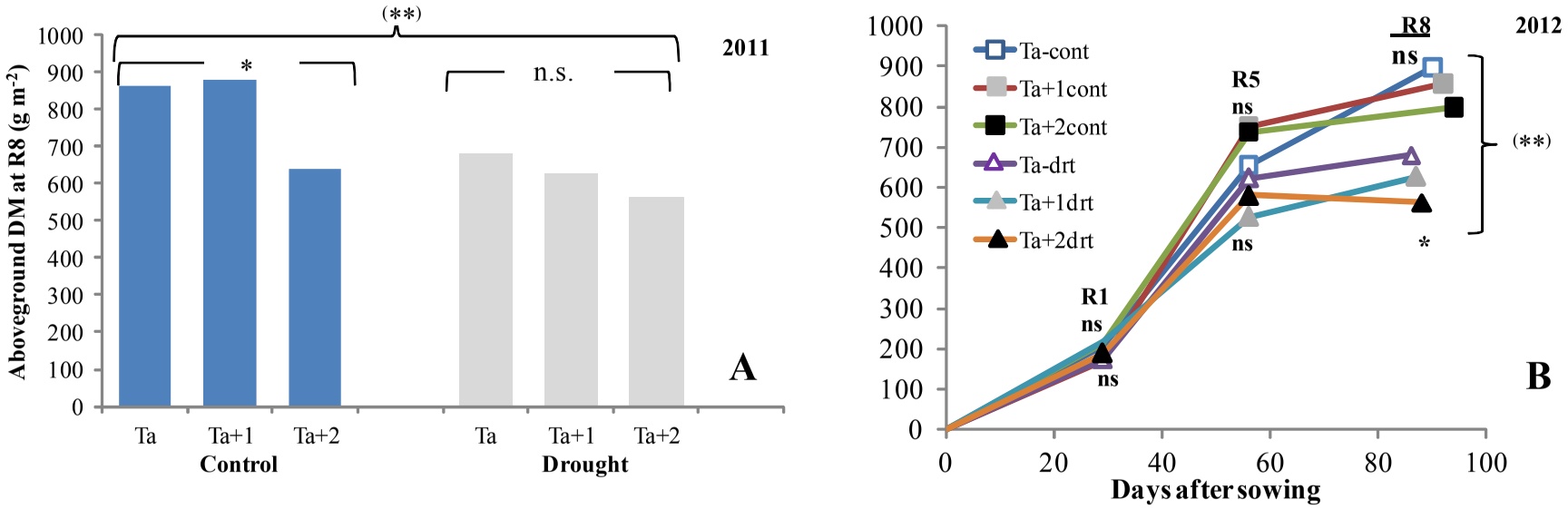

Figure 14. Temporal changes in aboveground dry matter of soybean cv. Enrei in 2011 (A) and 2012 (B) under well-watered (cont) and drought (drt) conditions. The n.s. and * displayed in the figures are for comparison between Ta and Ta+2 of each year and those in parenthesis are for comparison between the means of control and drought treatments.

4.3.4 Photosynthesis and stomatal conductance

In 2011, increased temperature by approximately 2°C resulted in reduced stomatal conductance and photosynthetic rates (chapter 3). In 2012, the photosynthesis response to increased temperature was similar in plants under drought stress and under well-watered, but the photosynthetic rates were always lower under stress conditions (Fig. 15).

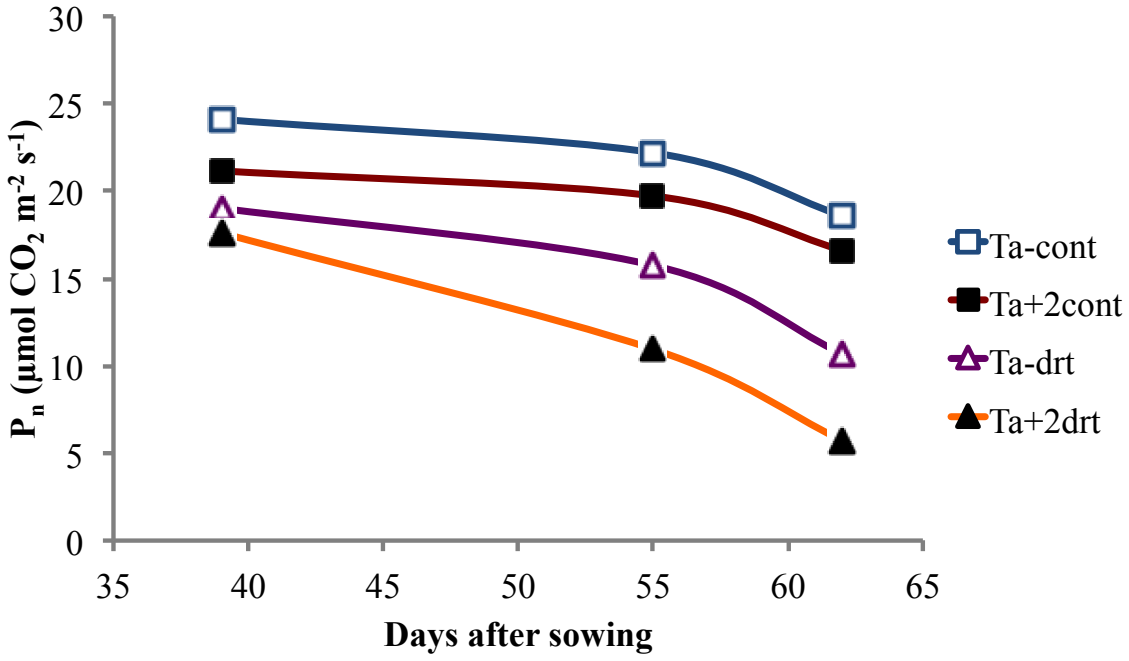

Figure 15. The response of photosynthesis and stomatal conductance to increased temperature under drought and well-watered condition in 2012.

4.3.5 Carbon Isotope Discrimination

CID ranged from 19.32 to 20.39‰ for seed samples and from 21.13 to 21.64‰ for leaf samples. The values were significantly lower under warmer treatments only for seed samples. The analysis of variance showed that there was no significant effect of water or its interaction with temperature on CID (Table 11).

Table 11. The response of carbon isotope discrimination to increased temperature in 2011 and 2012

Treatment		CID	
Water	Temperature	Seed (‰)	Leaf (‰)
Control	Ta	20.39	21.64
	Ta+2	19.78	21.13
Drought	Ta	19.86	21.55
	Ta+2	19.32	21.37
ANOVA		F value (p)	
Water (W)		2.97 (n.s.)	0.13 (n.s.)
Temperature (T)		4.00 (<0.1)	2.71 (n.s.)
WxT		2.25 (n.s.)	0.63 (n.s.)

4.4 Discussion

The 2^{o}C increase in temperature observed in most of the growing season had a considerable impact on plant development after flowering and have been discussed in chapters 2 and 3. Withholding water supply did not have significant influence on the onset of flowering as would be expected because water was withheld around flowering. However, as soil dehydration continued it shortened the duration of seed filling, which would be attributed to hastened N translocation from vegetative tissues associated with decline of N fixation and consequent acceleration of leaf senescence (Sinclair et al., 1987). Early onset of plant development from vegetative to reproductive in two soybean cultivars due to drought stress has been reported in a previous study (Desclaux and Roumet, 1996). In addition, reduced transpiration may have caused increased canopy temperature and hence faster development.

Increased temperature reduced seed yield in a similar manner described in chapter 2. Reduced fertile pods, reduced seed number and, to some extent, reduced seed size, either under high temperature or drought, resulted in low HI and low yield. High temperature is known to have negative impacts on reproductive processes of plants, including cereals (Barnabas et al., 2008) and legumes (Porch and Jahn, 2001; Koti et al., 2005; Hatfield et al., 2011). The sensitivity of reproductive stage to water stress has been reported in previous studies. Stress imposed during early pod formation had greatest reduction on soybean seed yield through reduced pod and seed number (Sionit and Kramer, 1977). Water stress early in the seed fill reduced the number of soybean seed per

pod (Desclaux et al., 2000), while even short episodes of water stress during seed filling resulted in reduced seed yield and smaller seeds (Brevedan and Egli, 2003).

Although they are commonly studied independently, the combined effects of abiotic stresses are known to be more detrimental than when applied individually (Mittler, 2006). The combined effects of high temperature and drought were greater than the additive effects of high temperature and drought alone on yield (Xu and Zhou, 2006; Pradhan et al., 2012), yield components (Prasad et al., 2011) and physiological parameters (Machado and Paulsen, 2001; Shah and Paulsen, 2003; Liu et al., 2008; Prasad et al., 2011).

In this study, the interaction of high temperature and drought, if any, would likely take place through stomata behavior and/or development of sink organs. When drought stress is developed, stomata tend to close and this results in higher leaf temperature and higher VPD between the leaves and atmosphere. Thus, with greater increase in VPD under warmer condition than in near ambient temperature, it would result in further stomatal closure. The results indicated, however, that the effects of high temperature and drought on each of yield and yield components were only additive.

One reason for the additive effects, despite the prolonged exposure to drought, might be the moderate drought. Although temperature and drought are likely to have synergistic effects, additive effects can occur when the stress levels are moderate, as reported for *Arabidopsis* (Vile et al., 2011).

4.5 Conclusion

In this study, conducted in field-like conditions, high temperature and drought affected seed yield and dry matter production of soybean. The impact on yield was the result of reduction of important yield components, including fertile pods, seed number and, to some extent, seed size, which affected the HI. The combined effects of high temperature and drought had additive effects on phenology and each of biomass and yield components, presumably due to moderate drought and relatively low temperature in some specific periods of reproductive stage. This response can be encountered in some warm regions of Japan, where moderate drought occur during the growing season of soybean, but it can be exacerbated if the drought is severe and prolonged as is predicted for the future climate.

Chapter 5

General discussion and conclusion

5.1 The effect of increased temperature on the processes of yield formation

With the mounting evidences that increase in the greenhouse gases will increase air temperature there are concerns among agricultural scientists, about the potential impacts of increased temperature on crop yield as temperature controls the growth and development in plants. Together with related areas such as genetics, plant breeding and biochemistry, crop physiology can play an important role in the development of management practices as well as improved cultivars that will help to cope with stressful environment. In this a detailed understanding of the physiological mechanisms by which plants respond to increasing temperature is needed.

The present study aimed at investigating the effects of increased temperature on soybean growth and seed production using a temperature gradient chamber that mimics the field conditions. A 2 to 3^{o}C increase in air temperature was observed in TGC, which affected the plant phenology, dry matter production and seed yield formation. In this study, increasing temperature delayed plant development, on the contrary of the commonly known effects of high temperature whereby temperature increase hastens the rate of development (Porter, 2005) and reduces the length of dry matter accumulation.

The progress of pod development appeared to be the most affected process of plant development and it had negative impacts on the final dry matter, harvest index and seed yield. The apparent disparity of results about the effects of increased temperature on plant phenology may reflect the differences in temperature optimum of cultivars used in each experiment, as was the case of Thomas et al. (2010) who found that a temperature above 26^{o}C also delayed the onset of reproductive process and seed formation of cv. Bragg. Another factor may be the fact that in most studies temperature was held constant throughout the entire experiment (e.g. Heinemann et al., 2006; Thomas et al., 2010) or during a specific development stage (Egli and Wardlaw, 1980; Gibson and Mullen, 1996) while in others temperature follows the diurnal pattern of ambient temperature (Thomas et al., 2010), which is the case of the current study using TGC. Plants subjected to varied fluctuating temperatures may experience periods of high temperature that coincide with sensitive developmental stages resulting in slower development and also may be exposed to the short-term occurrence of extremely high temperatrues. In this study plants were exposed to high temperature from the onset of flowering to early seed filling, therefore the pod set progress was the most affected.

This period of relatively high temperature was also the period where the major part of differences in dry matter accumulation occurred. At the harvest maturity aboveground dry matter was reduced by 9 to 26% and the extent of reduction was dependent on the degree of delayed senescence and the amount of abscised leaves. A peculiar aspect of TGC experiments, including this and previous studies in the same facility, is that increased temperature delayed the plant senescence. This phenomenon

was more pronounced in some years, especially in the warmest year of 2010 where at harvest maturity many leaves had abscised.

The seed yield was reduced by reduction of pod number and seed number (m^{-2}), and further reduction occurred through reduction of single seed weight. The response of TDM also was unexpectedly greater than expected from earlier studies (Baker et al., 1989; Koti et al., 2007). Therefore, the large reduction of pod and seed number would be attribuble to reduction of dry matter accumulation during the period when pod and seeds were produced.

The reduction of TDM was associated with decline of photosynthetic rate and stomatal conductance under warmer conditions. Analyses of change of vapor pressure deficit (VPD) and carbon isotope discrimination suggested that the concomitant increase of VPD with increased temperature exacerbated the temperature effects. In addition, reduced ambient CO_2 and low light intensity as the artifacts of the facility might have accounted for the great effect of temperature.

High temperature are known to cause flower and pod abortion, abnormal pollen formation and reduced pollen viability. Temperature increase in this study might have a direct effect on yield through reduction of pollen viability and pod set ratio (Fig. 16).

In the current study the mechanisms by which single seed weight is affected by increased temperature were studied and the results indicated that the seed size is determined by seed growth rate, which in turn is dependent on cotyledon cell number. Despite the commonly reported lesser contribution of seed size in the final yield as compared with the number of seeds per unit area (Board and Modali, 2005), the reduction in seed size appeared to have caused further reduction in the seed yield (Fig. 16)

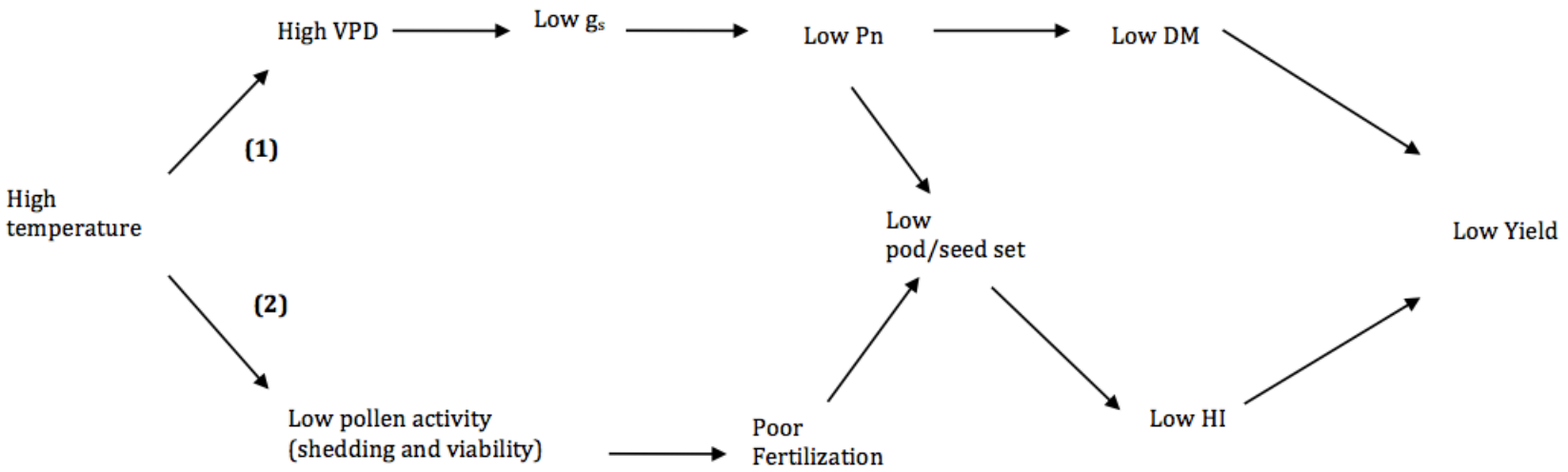

Figure 16. Schematic representation of DM and yield response to increased temperature under TGC conditions.

Increased temperature in TGC might have affected yield through reduced photosynthetic activity (route (1)) or through reproductive failure (route (2)) (Fig. 16). In TGC, unlike in many controlled environments, the relative humidity is not controlled and this results in increased vapor pressure deficit (VPD) as temperature increases. The increased VPD, sometimes over 2 kPa during the day, caused earlier stomatal closure which resulted in reduced stomatal conductance (g_s) and carbon assimilation (Pn). As photosynthesis is reduced, the DM production and the final seed yield are also decreased. On the other hand, relatively high temperatures during the time of anther dehiscence might have affected pollen germination and pollen tube growth, resulting in poor fertilization and poor pod/seed set. This decrease in reproductive sink strength for assimilates might have resulted in lower HI and reduced final seed yield. Although the possibility of yield reduction through route (2) remains a matter for further studies, it was clear that increased temperature affects the yield formation process (Fig. 18).

5.2 Linking the results to the future climate

Temperature-related reductions of dry matter production and seed yield are likely to occur in the warm regions, including temperate zones in Japan. According to climate statistics by Japan Meteorological Agency (http://www.jma.go.jp/jma/indexe.html, Fig. 17), estimated values of VPD show that, although the minimum and the mean VPD have been relatively stable in the past decades, the maximum VPD has been increasing in the during the summer in Kyoto. This increase in VPD creates a high evaporative demand and may have consequences on gas exchange rate and dry matter production.

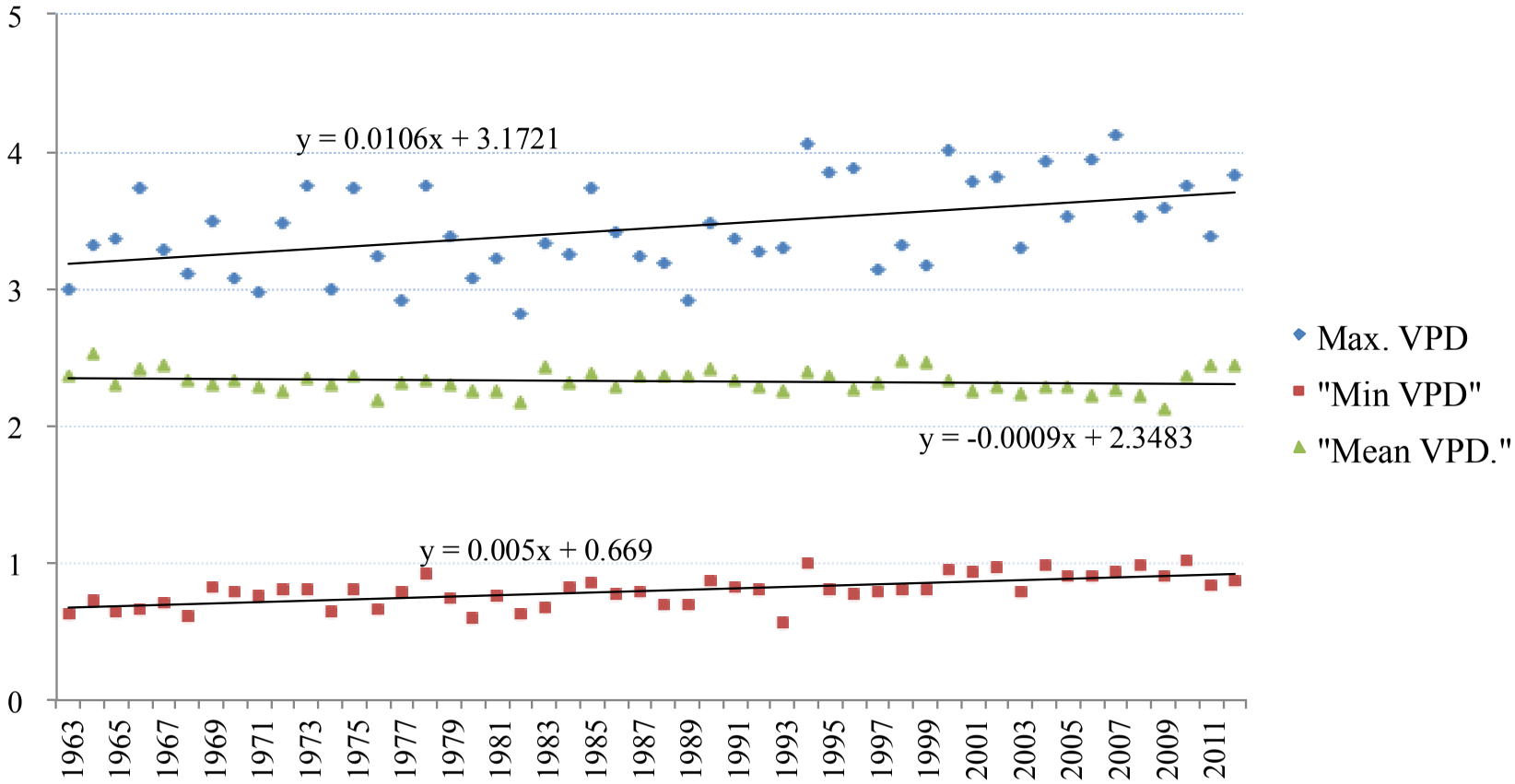

Figure 17. Estimated Max, Mean and Min VPD in Kyoto (Climate statistics by Japan Meteorological Agency: http://www.jma.go.jp/jma/indexe.html).

A consistent trend of dry matter production, HI and seed yield as affected by increasing temperature was observed when combining data from the four study years (Fig. 18). This suggests that indeed there is an optimum temperature above which dry matter production and partitioning are affected by increased temperature.

Figure 19 shows combined data from the current study and Tohoku Agricultural Research Center (Kumagai et al., 2011), a cool region in northern Japan. This suggests that for the cultivar used in the current study (Enrei), the temperature optima for HI of the cultivar used in the current study (Enrei) for the whole growth period is about 26^{o}C. Estimated temperature effects from the derived equation, show that a temperature increase of 2^{o}C above the optimum would reduce HI by 7%, whereas increasing temperature by 3^{o}C would result in 13% reduction.

With necessary caution when interpreting these results regarding the artifacts associated with the facility, the response from TGC conditions mimic the performance of soybean in future environment in warm regions of temperate zones.

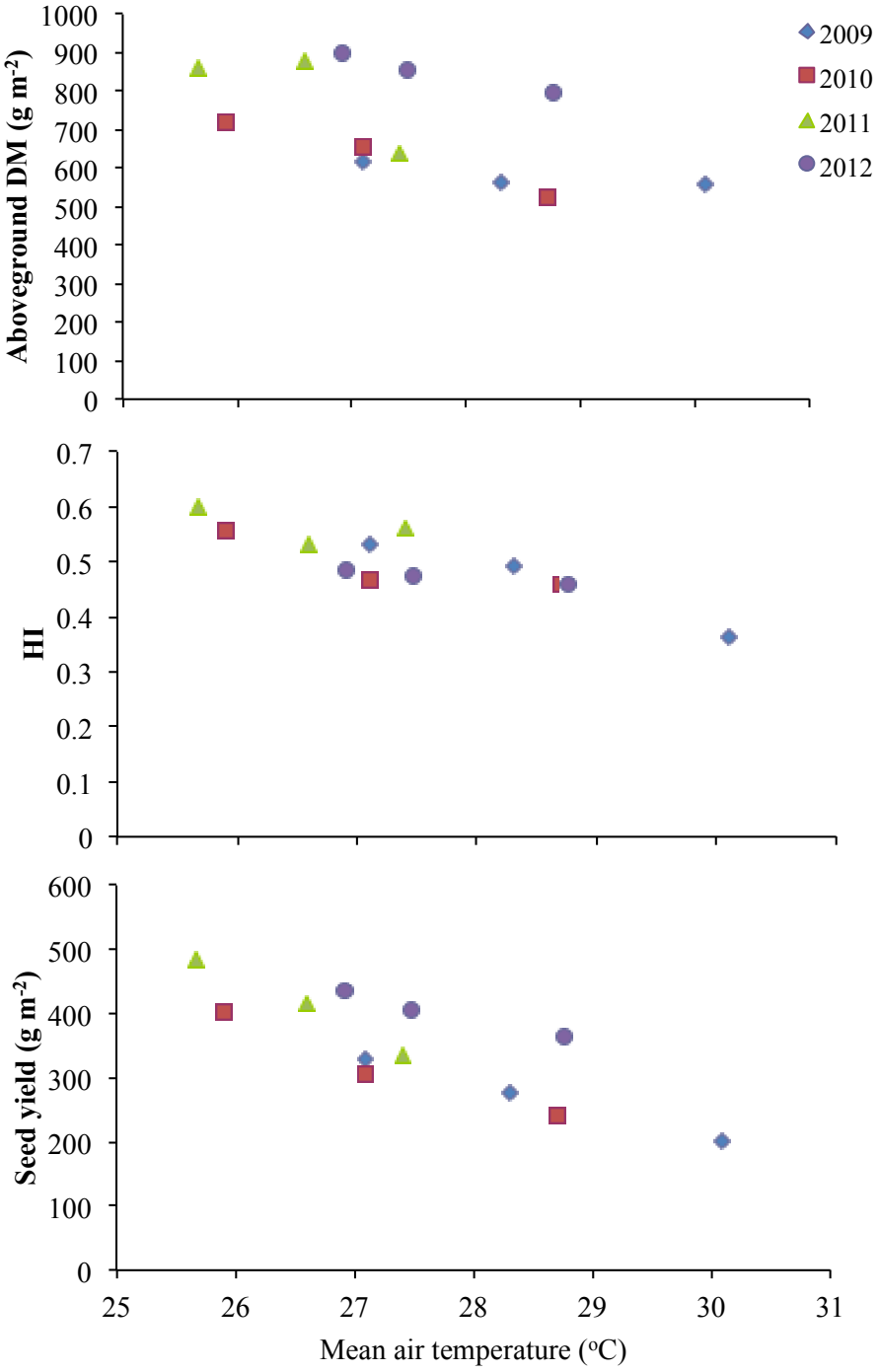

Figure 18. Total dry matter (DM), harvest index (HI) and seed yield as affected by temperature. Results from four years are combined. The results from the drought treatment are not included.

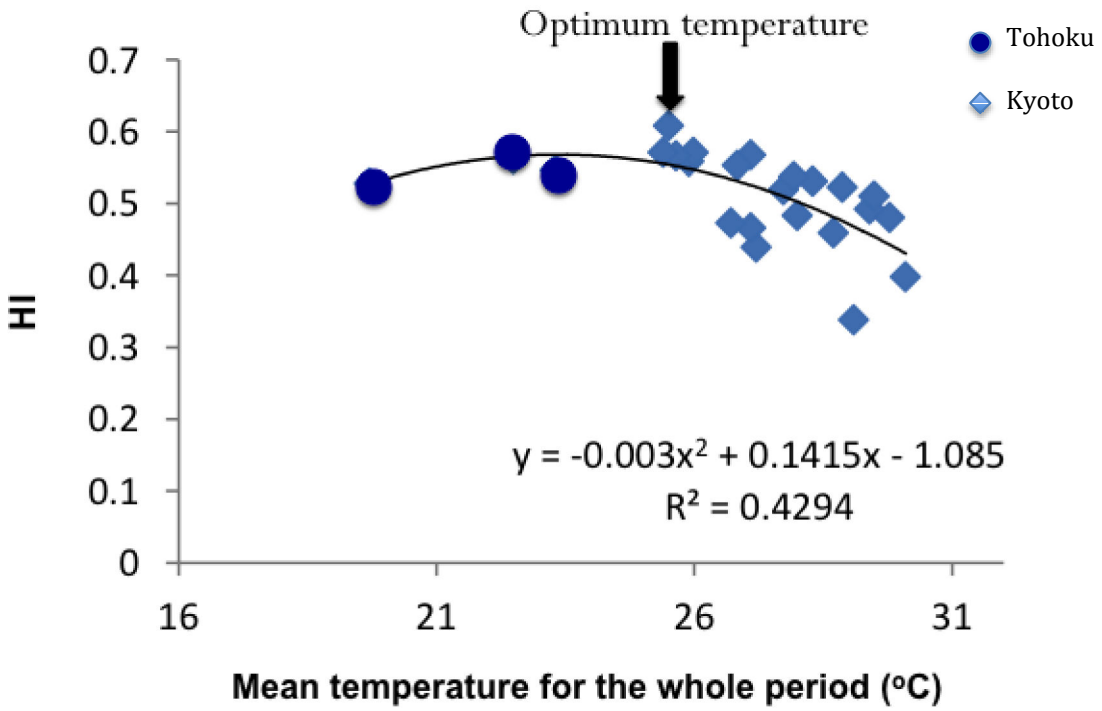

Figure 19. HI response to increased temperature in soybean cv. Enrei. (Combined data from the TGC studies conducted at Kyoto (this study) and Tohoku (Kumagai et al., 2011).

5.3 Conclusions

The impact of projected increase in temperature on soybean yield has been a subject of many studies around the globe but quantitative effects of increased temperature on soybean growth and seed production under field-like conditions are limited. Large effects of an increase in temperature by a few degrees were observed in this TGC study conducted under field-like conditions at Kyoto, a warm region of Japan. The yield was reduced in plants treated with an increase in temperature through reductions of pod and seed numbers associated with a decline in DM production. It was suggested that the reduction in ambient [CO_2] and light intensity and the concomitant increase of VPD with increased temperature in the TGC exacerbated the effects of increased temperature. The temperature-related reduction in yield is likely to occur in warm regions, where high temperatures coincide with atmosphere dryness and it might occur more often with the climate change; however, to what extent the dryness of the atmosphere exacerbates the effects of high temperature through drought is a matter for further studies.

Given that the global warming is projected to continue, adjusting cropping system not to expose plants to hot environments in their sensitive stages as well as breeding cultivars for high temperature environment will help plants cope with the warming climate.

Acknowledgements

This Thesis was completed after five years of study at the Laboratory of Crop Science, Graduate School of Agriculture, Kyoto University. I would like to express my appreciation to people who direct or indirectly contributed to the completion of this Thesis.

First of all, I would like to express my deepest appreciation and gratitude to Professor Tatsuhiko Shirawa for his guidance, useful comments and suggestions throughout the study. His enthusiastic approach, encouragement and persistent support played a crucial role in this accomplishment.

I would like to extend my gratitude to Dr. Koki Homma for his helpful assistance, discussion and suggestions during the course of this study. I am also grateful to Dr. Tomoyuki Katsube-Tanaka and Dr. Yu Tanaka for their valuable advice and comments on the research.

I would like to thank all members of laboratory of crop science, in special Mr. Yuichiro Yokomine, Ms. Sonia Hossain, Mr. Keiichiro Nakashima and Mr. Satoshi Nakano for the cooperation in the field.

I am grateful to Dr. Etsushi Kumagai and Dr. Ryoji Sameshima from Tohoku Research Center for their cooperation in the Project.

I would also like to thank Dr. Miki Sugimoto from the Laboratory of Animal Physiology and Functional Anatomy for helping in cell counting.

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List of symbols and abbreviations

Symbols/ abbreviation	Description
CID	carbon isotope discrimination
DAS	days after sowing
DM	above ground dry matter
EFP	effective seed filling period
g_s	stomatal conductance
HI	harvest index
Pn	Leaf photosynthetic rate
R1	beginning of flowering
R5	beginning of seed filling
R7	physiological maturity
SGR	individual seed growth rate
Ta	near ambient temperature
Ta+1	ambient temperature + 1^{o}C
Ta+2	ambient temperature + 2^{o}C
Ta+3	ambient temperature + 3^{o}C
TDM	total above ground dry matter
TGC	temperature gradient chamber
VPD	vapor pressure deficit

List of publications

(Chapter 2)

Tacarindua, C. R. P., Shiraiwa, T., Homma, K., Kumagai, E., Sameshima, R. The response of soybean seed growth characteristics to increased temperature under near-field conditions in a temperature gradient chamber. Field Crop Res. 131: 26-31 (2012).

(Chapters 2 and 3)

Tacarindua, C. R. P., Shiraiwa, T., Homma, K., Kumagai, E., Sameshima, R. The effects of increased temperature on crop growth and yield of soybean grown in a temperature gradient chamber. Field Crop Res. (2013, *in press*).

(Chapter 4)

Tacarindua, C. R. P., Shiraiwa, T., Homma, K. Interactive effects of increased temperature and water deficit on crop growth and yield of soybean grown in the temperature gradient chamber. (Under submission)